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A search for homologues of plant photoreceptor genes and their signaling partners in the sugarcane expressed sequence tag (Sucest) database

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Abstract

A search in the sugarcane expressed sequence tag (SUCEST) database for homologues of plant genes involved in photo-sensory mechanisms was carried out using the basic local alignment tool (BLAST). Our results shown that known elements (phytochromes, cryptochromes and phototoprin) present in *Arabidopsis* and other higher plants were detected with low e-values. We also searched for proteins interacting with photoreceptors in primary or downstream signaling events. One putative homologue for a protein postulated to be a primary element in phytochrome signaling pathways was identified, as were other candidates for downstream interacting factors.

INTRODUCTION

Extensive reviews are available focusing on all aspects of photoreception in plants (Nagy *et al.*, 2000; Quail, 2000; Hudson, 2000). However as the field is broad, we will present basic information for a better evaluation of our results.

The molecules engaged in the perception of light are specialized ones and are classified based upon the quality of the light absorb, *i.e.* phytochromes absorb in the red and far-red, and the cryptochromes and phototropin absorb UV-A and blue light.

In *Arabidopsis* the photoreceptors are present as two groups: the first group being the phytochrome gene family, which has five members (phytochromes A, B, C, D and E) and the other group consisting of two cryptochromes (cry1 and cry2) and one phototropin (nph1).

Phytochromes are photochromic pigments that exert their regulatory activity by switching between two forms: an active Pfr form by absorbing red light and an inactive Pr form by absorbing far-red light (Figure 1). Phytochromes are localized in the cytosol in both etiolated seedlings and in plants adapted to growth in the dark. Subsequent nuclear residence of phytochromes depends on quality and quantity of light (Nagy *et al.*, 2000; Nagy *et al.*, 2001).

Three groups of researchers, utilizing the yeast two-hybrid system, were able to locate candidates for primary phytochrome action.

One primary phytochrome action candidate is phytochrome Interacting Factor 3 (PIF3) which was isolated by Peter Quail's group using as bait the portion of phytochrome B COOH-terminal domain which is not involved in chromophore binding response. Interestingly the re-conversion of phyB to its non-reactive form caused dissociation of PIF3. This result was the first *in vitro* demonstration of a photo-reversible binding between a phytochrome and its putative signaling partner (Ni *et al.*, 1999). According to Zhu *et al.* (2000) the apparent affinity of PIF3 is higher for phyB than for phytocheome A (phyA) PIF3 has a bipartite signature with a nucleus localization signal (NLS) and a basic helix-loop-lelix (bHLH) motif of this super-family of transcription factors, common in the *Arabidopsis* genome. The target DNA is a palindrome, CACGTG, of the G-box sequence motif, present in various light-regulated gene promoters (Martinez-Garcia, 2000). The nuclear localization of PIF3 and its binding properties indicate that after translocation to the nucleus the photoactive form of phyA or/and B, interacts with PIF3; this being a primary stage in light-induction gene activation. The overall situation is thus a positive regulation of phytochrome action.

A second primary phytochrome action candidate was isolated using the C terminal domain of PHYA as bait by Joanne Chory's group who isolated a protein with different properties, phytochrome kinase substrate 1 (PKS1) (Fankhauser *et al.*, 1999) present in the cytoplasm. The N-terminal of this protein can be phosphorylated on its serine and threonine residues by preparations of recombinant oat phyA extracted from yeast, in Pr and Pfr conditions, but not with the protein obtained from dark grown controls. It thus appears that phyA function as a protein-kinase. This data supports the proposition that PKS1 is a candidate for a primary partner in phytochrome signal transduction initiation. A scheme for the putative interactions of phytochromes, PKS1 and PIF3 is shown in Figure 1

The third possible primary phytochrome action candidate, isolated by Choi *et al.* (1999) is nucleoside diphosphate kinase 2 (NDPK2). However NDKP2 is known to participate in several other developmental processes in

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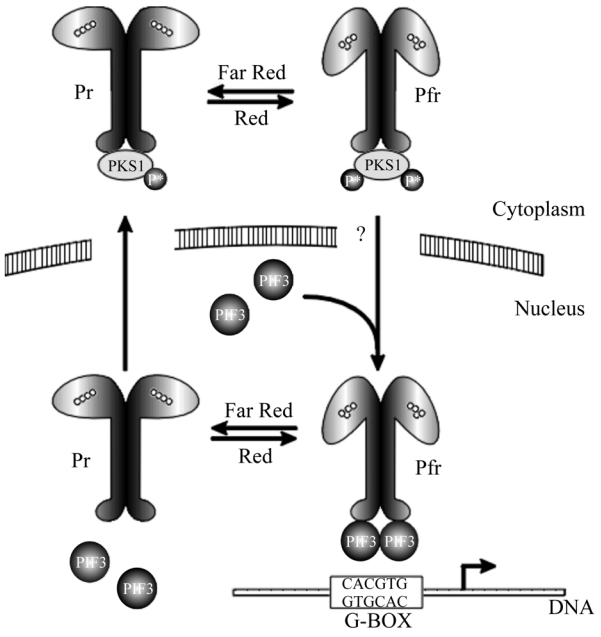

Figure 1 - The scheme (modified from Smith, 1999) is based on the data from Quail's and Chory's groups on PIF3 and PKS1. Phytochromes are represented by their dimeric form. They oscillate between an **inactive** state (**Pr)** and an **activated** (**Pfr)** form, under far-red or red light, respectively. The mechanism which govern the nucleo-cytoplasmic partitioning of phytochromes are of limited information in higher plants. PKS1 has two possible levels of phosphorylation and may act as a docking protein in the less phosphorylated state. The possibility of PIF3 associating with other transcription factors that also recognize the G box motif has been suggested. The symbol oooo represents tetra-pyrrol - P phosphorylated residue in PKS1 (see text).

other organisms and the present data in plants is far from conclusive.

Several other transcription factors are shown to act downstream in photoreceptor transduction pathways, *e.g.* the bZIP (basic Zipper) circadian clock associated 1 (CCA1) (Wang *et al.* 1998), the nuclear protein GI (GIGANTEA) (Huq *et al.*, 2000), the bHLH protein, long hypocotyl in far-red (HFR1) (which binds PIF3 by a possible mechanism of in vivo heterodimerization (Fairchild *et al.*, 2000) and COP1 (constitutive photomorphogenic 1), a negative regulator of CRY1 (McNellis *et al.* 1994) (Yang *et al.*, 2001).

Three blue light photoreceptors, cryptochrome1 (cry1), cryptochrome 2 (cry2) and phototropin (nph1) were identified in *Arabidopsis*. The main regulatory events for this class of photoreceptors are, hypocotyl inhibition, flowering time and phototropism, while other functions include stimulation of cotyledon expansion and circadian changes in response to light.

Phototropism appears to be exclusive of nph1 regulation (Christie *et al.*, 1998; Liscum and Stowe-Evans, 2000) but the possibility is not excluded of other photoreceptors acting in the range of high intensity blue light. Recently it has been observed in *Arabidopsis* that a NPH1 homologue, the protein NPL1 seems to be functionally linked to the chloroplast high-light avoidance response (Kagawa *et al.*, 2001; Jarillo *et al.*, 2001). Unlike the position with phyA and phyB, little is known for downstream regulation for the blue receptors. In the case of phototropin (nph1), a candidate protein NPH3 (Moutchoulski and Liscum, 1999), with potential protein-protein interaction motifs was isolated and characterized by Moutchoulski and Liscum (1999).

Finally, although being proteins which reside in the nucleus (or are nuclear bound), interactions (cross-talk), collectively referred to as co-action, between phytochromes and cryptochromes, has been suggested by Guo *et al.* (2001), as is the case for the Ca^{2+} binding protein SUB1.

MATERIAL AND METHODS

All sequences analyzed were from the SUCEST database. Descriptions of the libraries that generated the Ests clones as well as protocols for sequencing and the general strategies used in the SUCEST project can be found at http://sucest.lbi.dcc.unicamp.br. Sequence analysis was performed using the BLAST facility and the `search by keyword' service, both available on the SUCEST site, and the clustalX 1.8 program (Thompson *et al.*, 1997) on a workstation running under Linux (Debian 3.0) with the ToolKit 6.1 (NCBI), Vibrant 6.1 (NCBI) packages installed.

The BLAST program used was tblastn, using as query sequences proteins mainly from *Arabidopsis thaliana* and *Oryza sativa* genomes. The search by keyword service on the SUCEST site uses as database the Blastx and Blastn of the SUCEST sequences in NCBI Blast site response. The results were filtered restricting the hits to an E value 1E-15. The phylogenetic dendrogram was constructed using ClustalX 1.8.

RESULTS

Our search for homologues of genes involved in photoreception was focused on three main groups: 1) phytochromes, 2) cryptochromes and phototropin and 3) primary targets and downstream putative regulators. The Table1 is constructed in relation to the best score and lowest e-value.

PHYLOGENETIC RELATIONSHIPS

The grass family (POACEAE) has recently been submitted to extensive phylogenetic analysis of phytochrome B (*PHYB*) nuclear DNA sequences using a 1.2 kilobase (kb) region of the *PHYB* gene first exon that encompasses the chromophore binding site (Mathews *et al.*, 2000). Unfortunately, the complete sequence of this gene is not in the Sucest database. Nevertheless we applied the clustralX alignment program using the existent sugarcane *PHYB* sequence, against several complete sequences from current databases. The results are presented in the dendrogram (Figure 2).

Table I - Results of a Basic local alignment search tool (Blast) for groups of genes involved in photoreception. The sugarcane expressed sequence tag (Sucest) was used for the search. Clusters were made with the sugarcane expressed sequence tag (SUCEST) database and using the CAP3 (Contig Assembly Program, version 3).

Query sequence	Accenssion number	Sugarcane cluster	Score	E-Value
Phytochrome A				
Sorghum bicolor	GI11134026	SCCCCL3080H06.g	1439	0.0
Zea mays	GI130186	SCAGAM2017G02.g	325	2E-88
Phytochrome B				
Sorghum bicolor	GI11134029	SCQSLR1040D12.g	708	0.0
Phytochrome C				
Sorghum bicolor	GI11134032	SCCCLR1065C10.g	903	0.0
Cryptocrome 1				
Oryza sativa		SCAGST3138B05.g	421	1E-117
	GI16444957	SCJLHR1027C08.g	544	1E-154
		SCBFRZ3009A01.g	308	2E-83
Cryptochrome 2				
Lycopersicon esculentum	GI8101444	SCRFST1042F05.g	270	2E-71
Phototropin				
Zea mays	GI2687358	SCCCRZ3001D06.g	1009	0.0
PIF3				
Arabidopsis thaliana	GI3929585	SCCCRZ2001E12.g	178	2E-045
		SCEPAM2013E03.g	131	1E-030
NPH3				
Arabidopsis thaliana	GI6224711	SCQSST1038C03.g	381	1E-105
		SCEQAD1018D12.g	326	3E-89
CCA1				
Arabidopsis thaliana		SCCCLR1048E10.g	134	2E-31
	GI15226444	SCEZSB1093G09.g	100	1E-20
		SCEQRT2027F03.g	105	3E-22
COP1				
Oryza sativa subsp. indica	GI13925701	SCCCCL6003C08.g	618	1E-176
Oryza sativa subsp. indica	GI13925701	SCJFRZ2013A10.g	200	2E-50
GIGANTEA				
Oryza sativa	GI5912299	SCCCRZ1001A06.g	1390	0.0
Arabidopsis thaliana	GI6002520	SCCCRZ3001B05.g	225	4E-58
Arabidopsis thaliana	GI17433083	SCVPRT2079D03.g	281	1E-74
SUB1				
Arabidopsis thaliana	AL161512	SCCCLR2001G03.g	529	1E-149

DISCUSSION

The sequencing of the genome of the weed *Arabidopsis thaliana* during the Arabidopsis Genome Initiative reveal novel information about genomic organization, plant physiology and evolutionary relationships. One aspect with implications for some general conserved mechanisms between plants was the high degree of gene redundancy for several proteins of known and unknown function.

This peculiar aspect of the *Arabidopsis* genome seems to be represented in the small family of phytochromes (A to E) known to be present in this plant. Mutant forms of phyA and phyB are frequent (see the review by

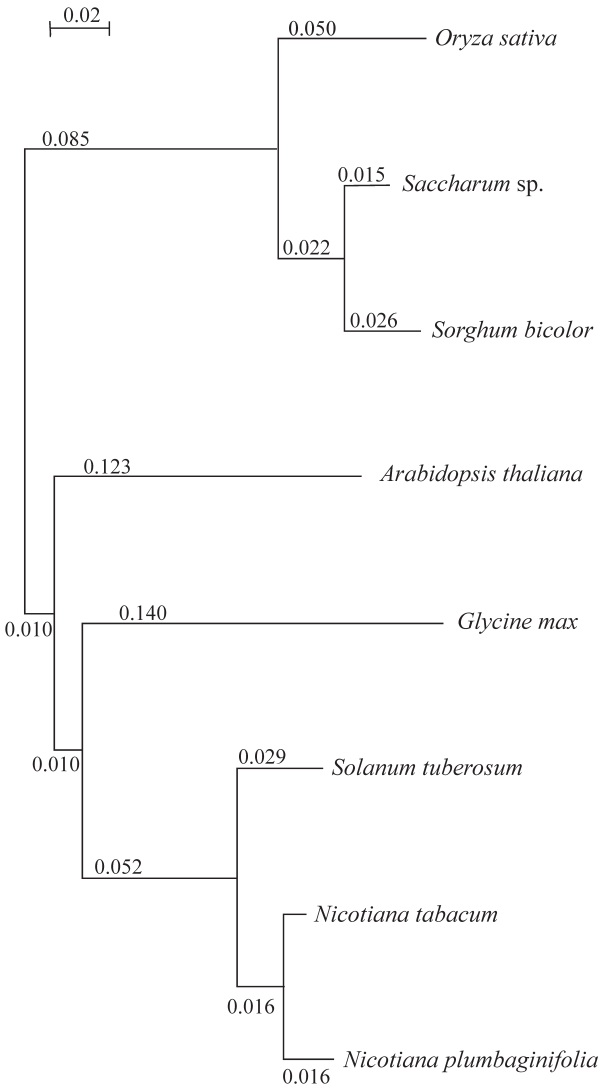

Figure 2 - Partial phylogenetic comparisons between the *PHYB* sequence from *Saccharum sp* and complete *PHYB* sequences available for other plants.

Hudson, 2000), but phyC and phy E mutants remain elusive. Only one natural null mutant of phy D has been observed which indicates a minor role for this phytochrome. Why then are phyD and phyE maintained if most of their functions are noticeable only in a *phyA/phyB* double-mutant background? Hudson has suggested that "the redundancy that they provide may be the key for their retention, rather than any specific response for which they are required in wild type *Arabidopsis*".

We are able to detect phyA, phyB and phyC homologues in the SUCEST database. This is the same situation observed in rice. In this monocotyledon it was possible to demonstrate that the *PHYA*, *PHYB* and *PHYC* genes are all located on chromosome 3, and of conserved synteny with these same genes in linkage group C from *Sorghum* (Basu *et al.*, 2000).

This redundancy or high gene density seems to be a recurrent model in small and large grass genomes (wheat, barley and rice) as is the case for the *Lrk* genes that encode receptor-like kinases (Feuillet and Keller, 1999).

The present identification (by sequence homology) of at least three members of the phytochrome family in sugarcane, as well as homologues to cryptochromes and phototropin will open the possibility of interesting comparisons at a genomic level to other monocotyledons. In this sense it is noteworthy that, as observed for maize, no NPL1 (phototropin homologue) was found in the SUCEST database.

With the exception of PKS1, not found in the SUCEST database we found homologues to genes being postulated to act in the primary photoreceptor response, mainly PIF3. However in the case of NDPK2, several other putative sequences for NDPKs were present which may suggest a more general role for this protein. Because of this, this element was not included in our table. In this context the conclusive presence of a PIF3 homologue reinforces the possible implication of this protein in primary phytochrome signaling in plants.

The situation is also very interesting for other members described previously as important elements in photo-transduction. High scores were found for CCA1 and GIGANTEA, with Table I giving a complete list of elements. The FHY1 protein, a recent identified phytochrome A-specific signal transducer was not found (Desnos *et al.* 2001).

Although incomplete, the dendrogram showed in Figure 2 aligned *Saccharum* with *Sorghum bicolor* but distant from *Oryza sativa*. The sequence available from *Zea mays* is incomplete and in a region not comparable to that from *Saccharum*. The information for maize is limited and we suggest that the completion of the phytochrome B sequence from either sugarcane or maize will furnish interesting phylogenetic relationships in relation to the position of these plants in the grass family (Mattews *et al.*, 2000).

Our studies have revealed basic evolutionary information and open the opportunity to apply to sugarcane the same approach that revealed essential information about light transduction signaling in other plants. Taken together, these results contribute valuable information to what is known about light transduction signaling in monocotyledons.

RESUMO

A partir dos dados do projeto de sequenciamento de Ests da Cana de Açúcar (Sucest/FAPESP) e utilizando BLAST (tblastn) como ferramenta, foi realizada uma busca de genes homólogos aos elementos envolvidos nos processos de foto-recepção e já descritos para outras plantas, principalmente *Arabidopsis*. Foram obtidas altas identidades para os fitocromos A, B e C assim como para os criptocromos 1, 2 e a fototropina. Diversos elementos identificados como reguladores primários ou secundários na

transdução de sinal de foto-receptores também foram identificados com baixos valores de E-value.

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