Research Article

Conserved Microsynteny of NPR1 with Genes Encoding a Signal Calmodulin-Binding Protein and a CK1-Class Protein Kinase in Beta vulgaris and Two Other Eudicots

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NPR1 is a gene of central importance in enabling plants to resist microbial attack. Therefore, knowledge of nearby genes is important for genome analysis and possibly for improving disease resistance. In this study, systematic DNA sequence analysis, gene annotation, and protein BLASTs were performed to determine genes near the NPR1 gene in Beta vulgaris L., Medicago truncatula Gaertn, and Populus trichocarpa Torr. & Gray, and to access predicted function. Microsynteny was discovered for NPR1 with genes CaMP, encoding a chloroplast-targeted signal calmodulin-binding protein, and CK1PK, a CK1-class protein kinase. Conserved microsynteny of NPR1, CaMP, and CK1PK in three diverse species of eudicots suggests maintenance during evolution by positive selection for close proximity. Perhaps close physical linkage contributes to coordinated expression of these particular genes that may control critically important processes including nuclear events and signal transduction.

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1. INTRODUCTION

Research done on Arabidopsis thaliana (L.) Heynth over a 10-year period in a number of laboratories has amassed considerable evidence that the NPR1 gene (also called NIM1) is of central importance in determining the plant ability to resist microbial attack [1]. In essence, global plant defense responses to pathogen invasion are controlled by the NPR1 gene product and intracellular redox state, since an inactive dimeric NPR1 protein in the cytosol is reduced to the active monomer which then migrates to the nucleus and activates expression of pathogen-induced "pathogenesis-related" (PR) genes [2].

The central role of NPR1 in positively activating defense mechanisms in response to biotic stress suggests the possibility of enhancing disease resistance in plants by genetic manipulation of the NPR1 gene. In fact, an increasing number of attempts to improve disease resistance in plants by modifying expression of NPR1 have been reported [3–7].

Microsynteny is genomics information that can be used to predict the location of homologous genes in different species. Knowledge of microsynteny of genes colinear with NPR1 in crop species could perhaps be used to devise innovative strategies for molecular genetic modification in order to improve disease resistance. As a step towards identifying genes located near the NPR1 gene in sugarbeet, a bacterial artificial chromosome (BAC) library [8] was screened and an NPR1-carrying clone, SBA091H24, was identified [9]. The B. vulgaris BvNPR1 gene encodes a predicted protein product being 100% identical to that deduced from the sequence of the cDNA for B. vulgaris NPR1 (GenBank accession AY640381). SMART analysis of the predicted BvNPR1 gene product [9] showed a BTB/POZ domain and two ARDs, or ankyrin repeat domains [10], both being characteristic of NPR1 proteins and other transcriptional activators within the nucleus. NPR1 is responsible for disease resistance priming or "induced resistance," a result of coordinated expression of multiple defense mechanisms/pathways to effectively resist microbial attack [11].

The NPR1 gene in sugarbeet has been only recently shown [12] to be essential for induced systemic resistance, as in the model A. thaliana (L.) Heynth [1]. In both
plant species, the activated form of NPR1 migrates into the nucleus and activates the transcription of genes involved with resisting disease-forming microbes [12].

Conservation of microsynteny among distinct families of eudicots was discovered in Lycopersicon esculentum Mill. (tomato) and A. thaliana, where large-scale duplications followed by selective gene loss have created a network of chromosomal synten— an accepted paradigm. By developing physical genetic maps based on expressed sequence tags (ESTs), Dominguez et al. [14] discovered conserved synteny with Arabidopsis among the genomes of four phylogenetically divergent eudicot crops, namely, sugarbeet, potato, sunflower, and plum.

In this study, complete BAC sequence analysis identified two core plant genes tightly physically linked to NPR1, and established a conservation of microsynteny between the NPR1 gene regions of sugarbeet and two other eudicot species. We report the gene content and organization of a 130 Kb DNA contig (continuous fragment) from an NPR1-carrying sugarbeet BAC. Comparison of similar NPR1-carrying DNA contigs from M. truncatula and P. trichocarpa showed that orthologs of genes encoding NPR1, a signal-peptide calmodulin-binding protein (CaMP) and a CK1-class dual-specificity protein kinase (CK1PK) occur in the same order and with a conserved direction of transcription in three divergent species of eudicots. This suggests positive natural selection for maintaining the physical proximity of genes whose products control certain essential nuclear events and a particular signal transduction function, as yet undefined.

2. MATERIALS AND METHODS

2.1. DNA sequencing

Genomic DNA of B. vulgaris hybrid US H20 [15], with an estimated 750 Mb genome size, had previously been used to construct a BAC library by ligating large DNA fragments resulting from partial HindIII digestion into vector pECBAC1 [16]. About 34,500 clones comprised the BAC library, and the average insert size was about 120 Kb, providing about 6.1X genome coverage [8]. Primers designed on the basis of data extracted from GenBank accession AY640381, a cDNA sequence for B. vulgaris NPR1, were utilized to screen and identify a BvNPR1-carrying BAC [9]. The presence of a complete genomic BvNPR1 gene was established by partial DNA sequence analysis of BAC clone SBA091H24 (GenBank accession DQ851167) [9].

BAC sequencing was completed at Washington University’s Genome Sequencing Center in St. Louis, Mo, USA (http://genome.wustl.edu/). The BAC clone SBA091H24 was provided to the Genome Sequencing Center as a glycerol stock. Purification, library construction, shotgun cloning, and sequence analysis were performed on a sufficient number of random subclones to provide about 9.4X coverage. ABI 3730 capillary sequencers were used. Data was assembled using the phred/phrap suite (http://www.phrap.org/).

2.2. Gene annotation

Analysis of the sequence data was performed using Lasergene (DNASTAR, Inc., Madison, WI, USA) for assembly and NCBI BLAST [17]. The sequence contig was screened for coding sequence using a combination of the following programs: GeneMark [18, 19] for eukaryotes (http://exon.gatech.edu/GeneMark/eukhmmer.cgi), GenScan (http://genes.mit.edu/GENSCAN.html), FGENESH (http://softberry.com/), and GRAIL (http://grail.lsd.ornl.gov/grail-exp/). In all cases, A. thaliana was chosen as a model, and default settings were used. BLASTP searches were performed at the National Center for Biotechnology Information (NCBI) website (http://www.ncbi.nlm.nih.gov/BLAST/). Percent identities and percent similarities were obtained using BLAST alignments. Manual curations of proteins was performed using Lasergene MegAlign and EditSeq sequence analysis software, where applicable simple modular architecture research tool (SMART) [20] database (http://smart.embl-heidelberg.de/) and Motif Scan (http://myhits.isb-sib.ch/cgi-bin/motif_scan) were used to identify protein domains and motifs, respectively. ARTEMIS (http://www.sanger.ac.uk/Software/Artemis/) was used to collate data and facilitate annotation.

Calmodulin target database (http://calcium.uhnres.utoronto.ca/ctdb/ctdb/home.html) was used to identify the most likely calmodulin-binding site in a peptide sequence. The hmmtop server (http://www.enzim.hu/hmmtop/) was used to predict transmembrane helices, and a signal peptide was detected using the SignalP 3.0 and TargetP 1.0 servers (http://www.cbs.dtu.dk/services/).


2.3. Identification of colinearity

BLAST searches were performed for protein products of the predicted ORFs of the B. vulgaris NPR1 BAC against databases for A. thaliana, M. truncatula, P. trichocarpa, and O. sativa L. ssp. japonica. High-scoring pairs (HSPs), the predicted protein products with highly significant matches, were considered as products of orthologous genes. Corresponding DNA regions are considered to be microcolinear when two or more orthologous genes are present in physical proximity, in the same order, and are transcribed in the same direction.

GenBank accession NC_008472.1 is representative of linkage group VI of P. trichocarpa [21], and the subsets of particular interest were coordinates from 8404895 to 8453314. GenBank accession AC124609 represents BAC clone mth229b13, a subset of chromosome 2 of M. truncatula (http://www.tigr.org/tigr-scripts/medicago/contig_location_association.pl?chromosome=2) [22, 23]. AC124609 (1...61740 bases) was extracted and compared to our B. vulgaris GenBank accession EF101866 (1...129695 bases).
3. RESULTS

A 130 Kb BAC designated SBA091H24, containing *B. vulgaris* chromosomal DNA, more specifically the sugar beet NPR1 gene [9], was sequenced and fully annotated (GenBank accession EF101866). The bioinformatics tools FGENESH, GeneMark, GenScan, and GRAIL were used as gene finders. Predicted gene names and predicted functions of deduced amino acid sequences, where possible, are presented in Table 1, and a visual representation of exon structure is shown in Figure 1. On the 130 Kb contig, a total of 17 potential open reading frames (ORFs), or protein coding regions, were identified. Only four out of the 17 open reading frames (ORFs) were predicted to produce protein products with high amino acid similarity to known products of core plant genes (Table 1). In addition to four core plant genes, the 130 Kb contig contains five retrotransposon genes, a transposon gene, and seven other genes whose products lacked a predicted function.

In addition to NPR1, another core plant gene, predicted on the 130 Kb sugar beet DNA contig from BAC clone SBA091H24, was composed of two exons that encode a heat shock factor (HSF) protein with a conserved DNA binding domain (*E* = 2e⁻²⁹) from amino acid positions 45 to 205. This HSF gene is located between the NPR1 gene and gene Hp1, encoding a hypothetical protein with some similarity to retrotransposon-encoded proteins. The HSF gene and NPR1 are transcribed in opposing directions. The predicted HSF protein has moderately high similarity (Table 1) to the protein product of *HSFA9*—a leaf pattern morphogenesis-controlling gene of sunflower, *Helianthus annuus* [24].

Beginning at about 70 Kb upstream of NPR1, another core plant gene encodes a calmodulin-binding protein (CaMP) that, as SMART analysis revealed, has an IQ domain.
Table 1: Predicted genes and their designated functions.

<table>
<thead>
<tr>
<th>Gene</th>
<th>Product length (a.a.)</th>
<th>Best BLAST Hit</th>
<th>E-value</th>
<th>Similarity</th>
<th>Designation</th>
</tr>
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<tr>
<td>Hp1</td>
<td>259</td>
<td>ABE85118</td>
<td>$5.0E-08$</td>
<td>49/101</td>
<td>Hypothetical</td>
</tr>
<tr>
<td>HSF (similar to HSF9)</td>
<td>337</td>
<td>AAM43804</td>
<td>$3.4E-47$</td>
<td>185/320</td>
<td>HSF transcription factor</td>
</tr>
<tr>
<td>NPR1</td>
<td>604</td>
<td>AAT57640</td>
<td>$0.0E+00$</td>
<td>604/604</td>
<td>NPR1 disease resistance</td>
</tr>
<tr>
<td>Hp2</td>
<td>259</td>
<td>none</td>
<td></td>
<td></td>
<td>Hypothetical</td>
</tr>
<tr>
<td>Integrase</td>
<td>1516</td>
<td>ABE91091</td>
<td>$0.0E+00$</td>
<td>903/1535</td>
<td>Integrase (copia type)</td>
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<tr>
<td>Hp3</td>
<td>403</td>
<td>ABD83280</td>
<td>$1.1E-124$</td>
<td>315/325</td>
<td>Unknown</td>
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<tr>
<td>Reverse transcriptase (RT)</td>
<td>1501</td>
<td>ABE83303</td>
<td>$0.0E+00$</td>
<td>996/1503</td>
<td>Gypsy-type retrotransposon</td>
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<td>Hp4</td>
<td>302</td>
<td>CAH67120</td>
<td>$1.0E-13$</td>
<td>78/177</td>
<td>Hypothetical</td>
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<td>Hp5</td>
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<td>ABD83301</td>
<td>$1.0E-09$</td>
<td>41/63</td>
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<td>none</td>
<td></td>
<td></td>
<td>Hypothetical</td>
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<tr>
<td>Hp7</td>
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<td>ABD83280</td>
<td>$4.1E-60$</td>
<td>186/282</td>
<td>Unknown</td>
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<td>Coe1 (transposon gene)</td>
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<td>604/956</td>
<td>Transposon</td>
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<tr>
<td>ORF2 of Coe1 (an integrase pseudogene)</td>
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<td>ABA95677</td>
<td>$4.0E-70$</td>
<td>177/298</td>
<td>Rvt2 domain integrase pseudogene</td>
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<td>Calmodulin-binding protein (CaMP)</td>
<td>525</td>
<td>NP_974673</td>
<td>$7.0E-161$</td>
<td>361/495</td>
<td>Signal-peptide calmodulin-binding protein</td>
</tr>
<tr>
<td>RT (reverse transcriptase)</td>
<td>198</td>
<td>ABF81417</td>
<td>$3.0E-37$</td>
<td>126/213</td>
<td>RT-like gene</td>
</tr>
<tr>
<td>CK1-class protein kinase (CK1PK)</td>
<td>473</td>
<td>BAB92346</td>
<td>$0.0E+00$</td>
<td>411/476</td>
<td>CK1-class dual-specificity protein kinase</td>
</tr>
</tbody>
</table>

GenBank accession number or protein ID of the best BLAST hit, followed by the E-value and percent (similar/total amino acids) similarity between the query and the best hit.

Designation based on the deductions possible via bioinformatics tools listed in Section 2. Functional classification based on the result of protein BLAST search.

NA: not applicable; putative function of the product was not identified.

Similar to FGENESH 21 [26].

from amino acid positions 131 to 153. Motif Scan suggested (1) an IMP dehydrogenase/GMP reductase domain at amino acids 121–444, (2) several protein kinase phosphorylation sites, and (3) an involucrin repeat at amino acids 206–215. The predicted protein product gave numerous BLAST hits to calmodulin-binding proteins. Calmodulin target database predicted amino acid positions 201–209 as the most likely calmodulin-binding site. Transmembrane helices were predicted at amino acid positions 337–353 by the hmmtop server, and the SignalP 3.0 server predicted a cleavage site in the N-terminal signal-peptide sequence between glutamine and lysine in amino acid positions 17 and 18. Thus, we deduced that the mature CaMP protein is 508 amino acids in length. The pro-CaMP was predicted as 525 amino acids in length, and the presence of a membrane-targeted N-terminal signal-peptide sequence suggests that peptid maturation occurs upon passage into or through a membrane. TargetP predicted that the targeted sites of subcellular localization of mature protein were chloroplasts.

Beginning at about 90 Kb upstream of NPR1, a third core plant gene encodes a “casein kinase class 1” protein kinase (CK1PK), identified by numerous BLAST hits with E-values of 0 to CK1PKs. SMART analysis revealed a dual-specificity STYKc protein kinase domain from amino acids 9–211. This type of kinase phosphorylates either serine, threonine, or tyrosine residues.

Conservation of microsynteny was discovered in B. vulgaris, M. truncatula, and P. trichocarpa (Figure 2), but not in A. thaliana or O. sativa L., by comparative DNA analysis of three core plant genes: (1) a CK1 class dual-specificity protein kinase gene, (2) a signal calmodulin-binding protein gene, and (3) the disease resistance-priming NPR1 gene. The high degree of amino acid similarity as well as identity of the predicted products of these respective microcolinear genes
(Table 2) clearly indicates that they are orthologous gene pairs with shared functions. The three orthologous gene pairs NPR1, CaMP, and CK1PK are colinear, that is, with both the same gene order and direction of transcription, in B. vulgaris, M. truncatula, and P. trichocarpa (Figure 2). In particular, in the comparison of presumptive orthologous gene pairs in B. vulgaris and M. truncatula, the NPR1, CaMP, and CK1PK genes encode proteins that have amino acid identities of about 60%, 57%, and 67%, and that exhibit amino acid similarities of about 74%, 68%, and 77%, respectively (Table 2). Similarly, in the comparison of presumptive orthologous gene pairs in B. vulgaris and P. trichocarpa, the three orthologous gene pairs, (NPR1, CaMP, and CK1PK), produce protein products with amino acid identities of about 65%, 56%, and 75% and amino acid similarities of about 79%, 68%, and 82%, respectively (Table 2). Finally, comparison of the amino acid identities and similarities of the predicted products of the NPR1, CaMP, and CK1PK genes in P. trichocarpa and M. truncatula (Table 2) indicates conserved orthologous gene pairs.

As mentioned above, about 2 Kb downstream of NPR1 in B. vulgaris, a fourth core plant gene (Figure 2) was predicted to produce a protein product with moderately high similarity to that produced by the embryonically expressed heat shock factor (HSF) A9 gene in sunflower. Microcolinearity of this particular HSF gene with NPR1 did not occur in M. truncatula. On the other hand, NPR1 in P. trichocarpa is separated from an HSF gene by only 1 Kb, but it encodes a protein that has only about 39% amino acid identity and 54% amino acid similarity with the protein encoded by the HSF gene adjacent to the NPR1 gene in B. vulgaris. Thus, the structure and function of the respective HSF proteins is not as highly conserved as were the three other colinear core plant genes in poplar and sugarbeet.

Comparison of the Arabidopsis NPR1 region with those of M. truncatula, P. trichocarpa, and B. vulgaris revealed that Arabidopsis lacks conserved microsynteny of CaMP and CK1PK genes with NPR1. In O. sativa L., the NPR1 genomic region has a gene encoding a calmodulin-binding protein, but it is transcribed in the same direction as NPR1 rather than in opposing directions as in B. vulgaris and M. truncatula; also the CK1PK gene, most proximal to NPR1 in O. sativa L., is greater than 250 Kb away (not shown). Thus, microsynteny, as in three out of four eudicot species, did not occur in O. sativa L., perhaps not unexpectedly as it is a monocot.

The six other noncore genes were retrotransposon or DNA transposon ones. Four putative genes were predicted to encode proteins with strong BLAST hits to retrotransposons or retrotransposon-like genes. An integrase gene has four exons and, from amino acid positions 750 to 900, this ORF encodes an rve core domain \(E = 3 \times 10^{-30}\), being a characteristic of integrases. With the integrase gene and the downstream putative gene Hp3 being combined, both share about 98% nucleotide identity (BLASTN) with highly related genes on a 9 Kb DNA contig (GenBank accession ABD83280) of BAC62 from sugarbeet chromosome 9 [26]. A putative reverse transcriptase gene about 5 Kb downstream of Hp3 consists of a single exon encoding a polyprotein with Rv12 RNA-dependent DNA polymerase domain \(E = 7 \times 10^{-24}\), an rve integrase domain \(E = 6 \times 10^{-21}\), and a poorly conserved \(E = 8 \times 10^{-10}\) gag capsid-like protein domain.

Coe1, previously identified by our group using LTR-STRUC analysis as a novel composite of class I and class II elements [25], has three genes. Briefly, one gene has a single exon ORF1 (Table 1), encoding a retroelement-like protein. ORF1 is about 2 Kb upstream of a gene that encodes a DNA transposase since its predicted product produced many significant BLAST alignments with DNA transposases, with
E-values equal to 0. The very highly conserved transposase family 
family \textit{tnp2} domain (\(E = 1 \times 10^{-64}\)) occurs in amino acid \newblock residue positions 200–400 of the predicted protein. \textit{Coel}’s \newblock transposase gene is also flanked by ORF2, a pseudogene of \newblock a \textit{copia}-like integrase, and all three genes are within LTRs \cite{7}. \newblock ORF2 is 2 Kb downstream of the \textit{tnp2}-class DNA transposase \newblock gene, and its predicted product showed, by BLAST analysis, \newblock a significant alignment with several putative retrotransposon \newblock proteins from wine grape and rice; for example, it aligned \newblock to \textit{O. sativa}’s putative \textit{Yl1-copia} subclass \textit{retrotransposon} (accession ABA95677) \newblock with \(E\)-value of \(\leq 1 \times 10^{-50}\), 38% \newblock amino acid identity, and 49% \newblock amino acid similarity.

Downstream of the gene encoding a calmodulin-binding \newblock protein, a putative reverse transcriptase gene encoded a \newblock predicted protein with only a moderate BLAST alignment \newblock (\(E = 3 \times 10^{-37}\)) to a reverse transcriptase \textit{Rvt-2} domain \newblock found in a novel \textit{retrotransposon}-like \textit{TIR-NBS-LRR}-type \newblock disease resistance protein in \textit{P. trichocarpa} Torr. & Gray \newblock (Table 1), and the similarity \newblock corresponds to a shared \textit{Rvt-2}-type \newblock domain.

In \textit{M. truncatula}, a \textit{TIR-NBS-LRR}-type resistance \newblock gene occurs downstream of \textit{NPR1} (not shown). The predicted \newblock product of the \textit{M. truncatula} resistance gene analogue (RGA) \newblock near \textit{NPR1} has 39% \newblock amino acid identity and 59% \newblock amino acid \newblock similarity with the \textit{Gro1-4} nematode resistance \newblock gene of \textit{Solanum tuberosum} \newblock (GenBank accession AY196152.1).

Comparative sequence analysis and gene annotation \newblock in \textit{B. vulgaris}, \textit{M. truncatula}, and \textit{P. trichocarpa} revealed \newblock that these three divergent species of eudicots exhibit \newblock conserved microsynteny of genes that encode the centrally \newblock important disease resistance priming \textit{NPR1}, a signal-peptide \newblock calmodulin-binding protein (CaMP), and a \textit{CK1}-class dual-specificity \newblock protein kinase (\textit{CK1PK}). The orthologs occur in \newblock the same order and with the same direction of transcription.

4. \textbf{DISCUSSION}

In this study, comparison of orthologous \textit{NPR1} gene regions \newblock of \textit{B. vulgaris}, \textit{M. truncatula}, and \textit{P. trichocarpa} revealed for \newblock the first time conserved microsynteny of the defense-priming \textit{NPR1} gene with a \textit{CaMP} gene, encoding a calmodulin-binding \newblock protein, and with a \textit{CK1PK} gene, specifying a \textit{CK1}-\newblock class dual-specificity \newblock protein kinase.

Calmodulin-binding proteins in plants are very diverse, \newblock exhibit various motifs, and perform a correspondingly wide \newblock variety of functions \cite{27–30}. For example, an \textit{Arabidopsis} \newblock ethylene-upregulated calmodulin-binding protein triggers \newblock senescence and death \cite{31}.

Calcium and calmodulin mediate a complex signal \newblock transduction network in plants through protein kinases \(newblock PKs), and some PKs are unique to plants \cite{32}. They are \newblock literal “hubs” of sensory perception and signal transduction. \newblock Examples include a calmodulin-binding PK in \textit{Arabidopsis} \newblock that negatively regulates tolerance to osmotic stress \cite{32} and \newblock a calmodulin-binding PK in tobacco \textit{(Nicotiana tabacum L.)}, \newblock involved with negative regulation of flowering \cite{33}.

It seems reasonable to hypothesize that the \textit{CaMP} gene product, \newblock which is a chloroplast-targeted, signal-peptide, \newblock calmodulin-binding transmembrane protein, could play a \newblock role in rapid activation of a defense cascade during either \newblock general stress or pathogen response. Just as the \textit{NPR1} \newblock gene is critical for disease resistance priming in plants, \newblock some calmodulin-binding proteins are pathogenesis-related. \newblock For example, \textit{de novo} synthesis of a calmodulin-binding \newblock peptide with a DNA-binding domain at the \textit{N}-terminus \newblock is induced by ethylene formed by the plant in response to \newblock wounding and/or infection \cite{34}; also in \textit{Arabidopsis}, \newblock another calmodulin-binding protein is pathogenesis-related \newblock as well \cite{35}. Moreover, in \textit{Glycine max} \newblock (soybean), specific \newblock calmodulin isoforms are required for the expression of \newblock pathogen-induced proteins upregulated by the \textit{NPR1} disease \newblock resistance control gene \cite{36}. Conserved microsynteny of \textit{CK1PK}, \textit{CaMP}, and \textit{NPR1} genes, \newblock discovered in three out of \newblock four eudicot species examined, \newblock could be hypothesized to \newblock suggest that their protein products play essential cellular roles \newblock related to plant defense response.

We propose a new hypothesis that conserved gene \newblock microsynteny of certain core plant genes in eudicots may \newblock correlate with either similar subcellular localization or with \newblock similar function. Either possibility for the protein products \newblock of the three core plant genes herein described is plausible. \newblock Activated monomeric \textit{NPR1} functions in the plant nucleus, \newblock where \textit{CK1PKs} are also localized. \textit{CK1s} are believed to \newblock control circadian rhythm \cite{37} and chromosome partitioning \newblock during meiotic cell division \cite{38} in all eukarotic cells. It \newblock should be noted that in \textit{O. sativa} a novel family of dual- \newblock specificity PKs is involved in controlling the plant responses \newblock to biotic as well as abiotic stresses \cite{39}. Based on available \newblock literature \cite{30, 31, 39}, the \textit{CK1PK} gene localized near \textit{NPR1} \newblock in certain eudicots may play a role in controlling \newblock the expression of stress-responsive genes in plants.

A total of 11 \newblock \textit{retrotransposons} (RTs), \newblock \textit{DNA transposons}, \newblock and hypothetical genes lie within the approximately 80 Kb

| Table 2: Percent amino acid identity and similarity exhibited by amino acid alignments of the conserved products of orthologous gene pairs. |
|---------------------------------|---------------------------------|---------------------------------|
| Orthologous gene pair          | \textit{B. vulgaris} \textit{NPR1} | \textit{B. vulgaris} \textit{CaMP} | \textit{B. vulgaris} \textit{CK1PK} |
| \textit{Medicago} homologs     | 60%/74%                           | 57%/68%                           | 67%/77%                           |
| \textit{Populus} homologs      | 65%/79%                           | 56%/68%                           | 75%/82%                           |
| \textit{Medicago} \textit{NPR1} | 62%/75%                           | 60%/71%                           | 69%/78%                           |
| \textit{Populus} homologs      |                                   |                                   |                                   |
stretch of DNA between the NPR1 and CaMP genes in B. vulgaris; therefore, we conclude that this region is rich in repetitive elements, and several insertions of mobile genetic elements have occurred during evolutionary divergence (Kuykendall et al., unpublished). ORFs, originating from either retrotransposons or viruses, from DNA transposons and other repetitive elements need not be considered disruptive of the conservation of colinearity when the core genes nevertheless occur in physical proximity, in the same order, and are transcribed in the same relative direction. Fortunately, our approach was not to be dissuaded by the repetitive elements that occur between NPR1 and CaMP in B. vulgaris comparing the orthologous regions of M. truncatula and P. trichocarpa, and thus we discovered conserved microsynteny for orthologous NPR1, CaMP, and CK1PK genes in these three eudicot species. This conserved microsynteny of NPR1, CaMP, and CK1PK in B. vulgaris, M. truncatula, and P. trichocarpa likely has an evolutionary basis through a yet undefined selective advantage.

The observed conservation of microsynteny of the three core plant genes could be hypothesized to result, in part, from positive natural selection for physical proximity. Hypothetically, close physical linkage may facilitate coordinated expression of genes critical in certain controlling nuclear events, such as those for which the protein products of CK1PK and NPR1 are known, or are responsible, either directly or indirectly, for the initiation of a dynamic signal transduction cascade as can be hypothesized for the predicted product of CaMP.

Reverse transcriptase (RT) PCR expression studies will be useful in determining whether the CaMP or CK1PK is upregulated in response to the administration of factors which induce the production of either pathogenesis-related or stress-related proteins. Such studies will hopefully allow one to determine whether the chloroplast-targeted signal-peptide calmodulin-binding protein gene (CaMP) or the nucleus localized casein kinase 1 protein kinase gene (CK1PK) may play a role(s) in pathogen and/or stress response.

In summary, in addition to NPR1 gene and the CaMP and CK1PK genes herein described for the first time, the 130 Kb NPR1-carrying B. vulgaris genomic DNA segment has 14 other features predicted by gene finders trained with Arabidopsis. Whereas seven ORFs produce predicted proteins with probable functions that can be deduced from BLAST analysis, seven other ORFs had predicted protein products without any known function.

It is also interesting to note that an HSF gene is located just 2 Kb downstream of NPR1 in sugarbeet, and the predicted product of this gene is a DNA-binding HSF protein similar to that specified by the HSF9 gene that controls early leaf morphogenesis in sunflower [24].

We conclude that conserved microsynteny of NPR1, CaMP, and CK1PK in three eudicot species suggests strong positive natural selection for the maintenance of physical linkage of these particular genes whose vital protein products either control specific nuclear events or are involved in signal transduction.

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REFERENCES


Special Issue on
Wet Laboratory Tools Widely Used in Plant Genomics

Call for Papers
There are numerous laboratory tools developed and available for use in plant genomics; however, they are usually published in books or in journal separate issues, making them accessed and updated inconveniently. Collection/integration of the wet laboratory tools that are widely used, further improved and advanced in a special issue that is open-accessed, is crucial to and greatly helpful for research of plant genomics and other disciplines of plant biology. The tools below listed themes are called for this special issue, but other tools that are useful for plant genomics are always welcome. The reviewed articles in other systems (e.g., mammalian system) that are potentially applicable to plant genomics are also considered for publication in the special issue. The emphasis and themes are as follows:

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• Molecular Tools
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  ◦ Methods for genetically modified organism analysis
  ◦ PCR methods (tail PCR, emulsion PCR, RACE-PCR, multiplex PCR, etc.)

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