

Fractionation of Synteny in a Genomic Region Containing Tandemly Duplicated Genes across *Glycine max*, *Medicago truncatula*, and *Arabidopsis thaliana*

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Abstract

Extended comparison of gene sequences found on homeologous soybean Bacterial Artificial Chromosomes to *Medicago truncatula* and *Arabidopsis thaliana* genomic sequences demonstrated a network of synteny within conserved regions interrupted by gene addition and/or deletions. Consolidation of gene order among all 3 species provides a picture of ancestral gene order. The observation supports a genome history of fractionation resulting from gene loss/addition and rearrangement. In all 3 species, clusters of *N*-hydroxycinnamoyl/benzoyltransferase genes were identified in tandemly duplicated clusters. Parsimony-based gene trees suggest that the genes within the arrays have independently undergone tandem duplication in each species.

Genetic colinearity and orthology has traditionally been identified through comparative mapping. Whereas marker colinearity has been highly conserved in the grasses (Ahn and Tanksley 1993; Devos and Gale 2000), in the legumes synteny has often been fragmented. Early marker studies identified conserved syntenic blocks among various legume genera, *Glycine max*, *Pisum sativum*, and *Vigna radiata* (Menancio-Hautea et al. 1993; Boutin et al. 1995). Choi, Kim et al. and Choi, Mun et al. (2004), using 60 mapped markers that were homologous between soybean and *Medicago truncatula*, identified 11 syntenic blocks. In these instances, synteny was limited to small intervals. Remnants of synteny were identified between soybean and *Arabidopsis* as well (Grant et al. 2000; Lee et al. 2001). A common theme of these studies is that synteny is high among the closely related species and decreases with larger phylogenetic distance. What has come to light in comparative studies in the grasses is that the conservation observed at the genetic map level is not necessarily observed at the sequence level (Bennetzen and Ramakrishna 2002; Feuillet and Keller 2002). In other words, genetic colinearity does not necessarily reflect sequence microcolinearity.

A BAC-based hybridization study between soybean and *Medicago* estimated that 27 of 50 soybean BACs possessed some level of microsynteny with *Medicago* (54%) (Yan et al. 2003). Yan et al. (2004) compared 8 groups of BACs between soybean and *Medicago* and found evidence of

synteny between 6 pairs and microsynteny between 3 pairs. More recently, an analysis of 3 megabases of soybean sequence showed unusually high synteny to 2 *Medicago* chromosomes with upward of 75% gene colinearity (Mudge et al. 2005). Cannon et al. (2006) conducted a large-scale sequence-based comparison between 2 cool-season legumes that are both members of the Hologalegina clade, *M. truncatula* and *Lotus japonicus*. Those authors reported that the 2 species shared a minimum of 10 large-scale synteny blocks with substantial colinearity.

Details of synteny between soybean (a member of the phaseoloides) and other legumes such as *Medicago* and *Lotus* as well as *Arabidopsis* are still largely undefined. In this study, genes from a genomic region defined by the presence of a tandemly duplicated *N*-hydroxycinnamoyl/benzoyltransferase (HCBT) gene family in soybean were compared with *Medicago*, *Lotus*, and *Arabidopsis*, 3 major dicot genomes with extensive sequence. Homologous regions in *Medicago* and *Arabidopsis* were identified revealing a network of synteny created by gene loss and rearrangement. This region was chosen because HCBT catalyzes the first committed step in phytoalexin biosynthesis (Yang et al. 1997), an important pathway involved in wound and pathogen response (Naoumkina et al. 2007). We also report that the HCBT gene clusters seem to have been created by independent tandem duplication in each species.

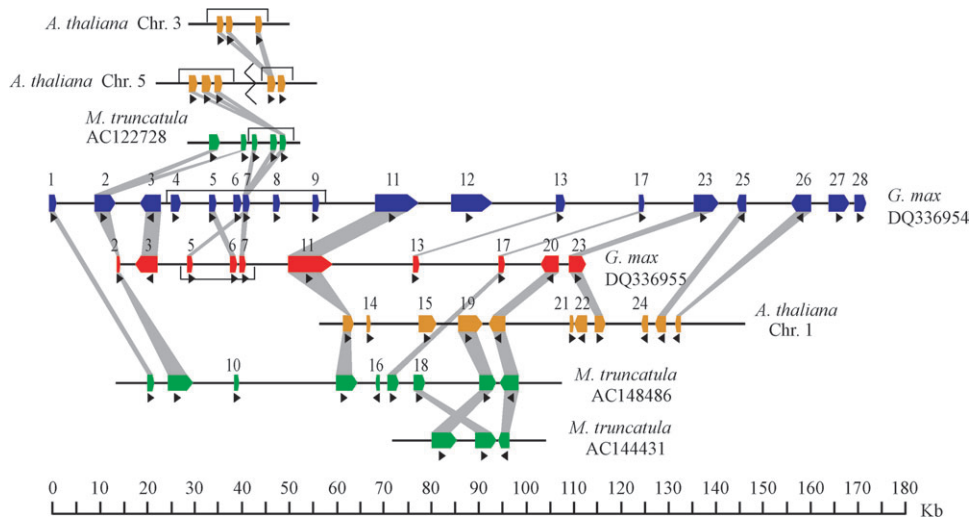


Figure 1. Diagrammatic representation of a network of synteny between 2 soybean homeologous regions, 3 *Medicago truncatula* BACs, and 3 *Arabidopsis thaliana* regions. Each colored arrow block represents a gene as numbered in Table 1. Gray bars between genes show the syntenic genes. The scale is in kilobases.

Materials and Methods

Previously, 2 homeologous soybean BACs, gmw1-74i13 (DQ336954) and gmw1-52d3 (DQ336955), anchored by HCBT were identified, sequenced, and annotated (Schlueter et al. 2006). Putative homologous sequences from *M. truncatula*, *L. japonicus*, and *Arabidopsis thaliana* were identified by performing a TBLASTX search with the annotated soybean gene structures against the available genomic sequences from *Lotus*, *Medicago*, and *Arabidopsis* at National Center for Biotechnology Information. Genes were considered homologues if their BLAST-based *e* values were less than 1×10^{-30} . Extended syntenic relationships were then identified by searching the resulting BAC or genomic regions for genes that were syntenic to other *Lotus*, *Medicago*, or *Arabidopsis* sequences.

Gene structures for *Medicago* were taken from MtGDB and further confirmed by GENESEQER alignments of all *Medicago* expressed sequence tags (ESTs) and all plant putatively unique sequences (consensus sequences derived from EST contigs) against each identified BAC sequence (Schlueter et al. 2003; 2006; Dong et al. 2005). Gene structures from *Arabidopsis* were taken directly from AtGDB (Zhu et al. 2003; Schlueter et al. 2006).

A multiple sequence alignment for all identified HCBT genes was generated with MEGALIGN (LASERGENE; DNASTar, Madison, WI) using default CLUSTALW alignment and manually removing large gaps due to single-sequence overhang at the 5'- and 3'-end of the alignments. A parsimony-based tree was generated in PAUP* 4.0 (Swofford 1998) with manual rooting with *Arabidopsis* as the out-group (internal node with a basal polytomy), tree bisection reconnection branch swapping, and 1000 iterations to generate bootstrap values.

Results

Identification of a Network of Synteny with *A. thaliana* and *M. truncatula*

Annotated genes from homeologous soybean BACs DQ336954 and DQ336955 were used to identify potential syntenic blocks in *Lotus*, *Medicago*, and *Arabidopsis* by searching with TBLASTX against the available genomic sequences for those species. No syntenic blocks were observed between soybean and *Lotus*. For each homologous gene from *Medicago* and *Arabidopsis*, the corresponding regions were scanned for blocks of synteny. Using this method, a network of synteny between the 3 species was identified (Figure 1).

An analysis of the structure of the chromosomal regions indicates that the regions have undergone gene additions and/or losses. Three syntenic blocks were identified in *Medicago* corresponding to BACs AC148486 from chromosome 1, AC144431 from chromosome 5, and AC122728 also from chromosome 5 (<http://www.Medicago.org>). The first BAC, AC148486, shares 4 genes (WUSCHEL-related homeobox [gene 1], poly-A-binding protein [PABP, gene 2], zinc finger [gene 11], and basic-helix-loop-helix [gene 17]) colinear in order and orientation with DQ336954 and DQ336955, respectively. AC148486 also contains a remorin gene (gene 20) found only on DQ336955 (Figure 1 and Table 1). However, this colinearity is fragmented with 4 other genes on AC148486 that are not found in the syntenic soybean regions, unknown (gene 10), glycosyl hydrolase (gene 16), S-ribonuclease-binding protein (gene 18), and lipase 3 (gene 19). It is through the S-ribonuclease-binding protein and lipase 3 that a fragmented network of synteny to the second *Medicago* BAC, AC144431, was identified (Figure 1 and Table 1). Beyond gene 20 on

Table 1. Syntenic genes between soybean, *Medicago*, and *Arabidopsis*

Gene	Putative function	Soybean DQ336954	Soybean DQ336955	<i>Medicago</i> AC148486	<i>Medicago</i> AC144431	<i>Arabidopsis</i>
1	WOX4 protein (homeobox-leucine zipper transcription factor protein)	Present		Present		
2	PABP	Present	Present	Present		
3	Membrane-like protein	Present	Present			
4	<i>N</i> -hydroxycinnamoyl/benzoyl transferase-like 1	Present				
5	<i>N</i> -hydroxycinnamoyl/benzoyl transferase-like 2	Present	Present			
6	<i>N</i> -hydroxycinnamoyl/benzoyl transferase-like 3	Present	Present			
7	<i>N</i> -hydroxycinnamoyl/benzoyl transferase-like 4	Present	Present			
8	<i>N</i> -hydroxycinnamoyl/benzoyl transferase-like 5	Present				
9	<i>N</i> -hydroxycinnamoyl/benzoyl transferase-like 6	Present				
10	Unknown protein			Present		
11	Zinc finger (C3HC4-type) 1	Present	Present	Present		At1g45180
12	Zinc finger (C3HC4-type) 2	Present				
13	Heat shock transcription factor	Present	Present			
14	<i>Arabidopsis</i> expressed protein					At1g45190
15	Glycosyl hydrolase					At1g45191
16	Reduced form of nicotinamide adenine dinucleotide dehydrogenase subunit fragment			Present		
17	Basic-helix-loop-helix transcription factor	Present	Present	Present		
18	S-ribonuclease-binding protein			Present	Present	
19	Lipase 3			Present	Present	At1g45201
20	Remorin-like protein		Present	Present	Present	At1g45207
21	Divergent C1 domain-containing protein					At1g45243
22	WW domain-containing protein					At1g45231
23	<i>Arabidopsis</i> -like expressed protein	Present	Present			At1g45233
24	<i>Arabidopsis</i> hypothetical protein					At1g45248
25	Basic leucine zipper transcription factor	Present				At1g45249
26	DCL protein	Present				At1g45230
27	Aromatic-rich family protein	Present				
28	Pentatricopeptide-repeat-containing protein	Present				

AC144431, no other genes were identified to be syntenic between these BACs. The final *Medicago* BAC identified, AC122728, contains a cluster of 3 HCBT genes and a gene encoding a PABP (gene 2) (Figure 1 and Table 1). No other HCBT genes with high sequence identity to those in soybean were identified from the available *Medicago* sequence.

Three chromosomes in *Arabidopsis* were found to contain sequences corresponding to sequences on the 2 soybean BACs (Figure 1 and Table 1). A region of chromosome 1 starting from At1g45180, a zinc finger protein (gene 11), to At1g45230, defective chloroplasts and leaves protein (gene 26), contains in total 11 genes, of which 5 are colinear in order and orientation with DQ336954 and 2 are colinear in order and orientation with DQ336955. Much as with the *Medicago* segments, the synteny is fragmented with noncolinear genes interrupting the colinearity to the soybean sequence.

The final 2 syntenic chromosomes in *Arabidopsis* contain only HCBT gene clusters as observed in soybean (Figure 1). The first cluster on chromosome 5 consists of At5g07850, At5g07860, and At5g07870; another region of chromosome 5 has At5g67150 and At5g67160. The chromosome 5 clusters are separated by just more than 24 Mb. On chromosome 3, another cluster of 3 HCBT genes, At3g50270, At3g50280, and At3g50300, was identified. No

other genes in this region of the *Arabidopsis* chromosomes are syntenic with either soybean or *Medicago*.

Three other copies of HCBT in *Arabidopsis* were identified (At5g42830, At2g39980, and At5g01210) but existed as single genes, not tandemly duplicated.

HCBT Divergence and Possible Independent Origins

Nucleotide diversity between HCBT genes within and between soybean BACs ranged from 59% to 84% (Schlueter et al. 2006). Within the *Medicago* clade, nucleotide identity ranges from 81% to 88%, whereas within the *Arabidopsis* clade, the HCBT genes showed greater divergence with identities ranging from 40% to 82%. Amino acid similarities ranged from 58% to 90% among soybean genes, 83% to 87% among *Medicago* genes, and 37% to 52% among *Arabidopsis* genes.

The multiple sequence alignment for all identified HCBT genes, using *Arabidopsis* as the out-group, generated a parsimony-based phylogenetic tree, which suggests that the tandem duplication of these genes likely occurred after speciation (Figure 2). The grouping of tandemly arrayed genes suggests that each cluster was independently formed. Genes from one species always form single clades (Figure 2). This is indicative of independent duplication events or may

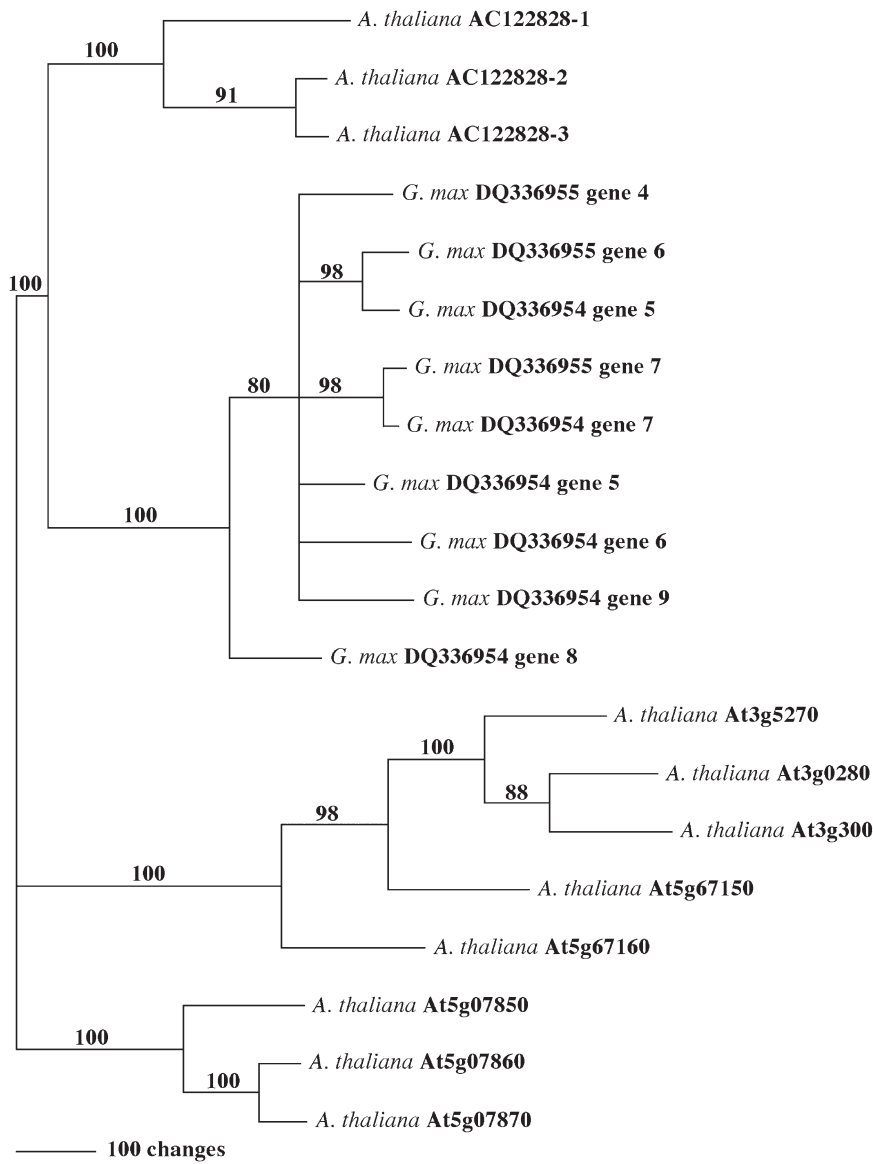


Figure 2. Parsimony-based gene tree of the tandemly duplicated HCBT genes from soybean, *Medicago*, and *Arabidopsis*. The resulting tree had a length of 3609, a consistency index (CI) of 0.566, a homoplasy index of 0.434, a retention index of 0.631, and a rescaled CI of 0.347. Bootstrap values are reported along corresponding branches based on 1000 iterations.

be the result of concerted evolution through gene conversion and gene loss through unequal crossing over.

Discussion

Of major interest to the legume community is the ability to utilize the developing sequences from the model legumes, *M. truncatula* and *L. japonicus*, as well as from *A. thaliana* for research in *G. max* and other phaseoloids. Particularly now that soybean is being sequenced via a whole-genome shotgun approach (Jackson et al. 2006), the possibility of using the BAC-by-BAC *Medicago* and/or *Lotus* sequence to aid in assembly of the soybean genome is an attractive

option. For this to be possible, however, sequence-based synteny between these legumes must be fairly strong. No syntenic blocks were identified from the available *L. japonicus* sequences. This may be due to sequences for these regions not yet available due to the ongoing process of genome sequencing or there might not be syntenic blocks in *Lotus* to these soybean sequences. The TBLASTX results (data not shown) suggest that the latter may be the case. Although 33% of the genes on DQ336954 had homologues in *Lotus* with an *e* value less than 1×10^{-30} , none of these genes were located on the same BACs. Marker-based studies have identified regions with upward of 54% synteny between soybean and *Medicago* (Yan et al. 2003; Choi, Kim et al. 2004; Choi, Mun et al. 2004). Previous work identified relatively

strong synteny between soybean and *Medicago* with up to 75% of the soybean genes colinear to *Medicago* (Mudge et al. 2005). This analysis shows that synteny between soybean and *Medicago* may not be significantly more conserved than between soybean and *Arabidopsis*, for some regions. This is somewhat surprising considering that the divergence of soybean and *Medicago* occurred approximately 50 million years ago (Lavin et al. 2005; Pfeil et al. 2005), whereas *Arabidopsis* and legumes likely diverged approximately 90 million years ago (Gandolfo et al. 1998; Yang et al. 1999; Grant et al. 2000).

Five of 9 genes contiguous on *Medicago* BAC AC148486 were conserved in order and orientation with at least one of the homeologous soybean BACs. However, 5 of 11 genes on a region of *Arabidopsis* chromosome 1 were also conserved in order and orientation with soybean. Although many of the genes that are conserved between species have the same order and orientation, synteny is fragmented between all 3 species. This suggests that there have been extensive additions and/or deletions of gene after divergence of these species.

These findings are somewhat different to those of Mudge et al. (2005) who found a region of hypersynteny between the 3 species. Although microsynteny may be used to identify orthologous regions from one legume species to another, based on our findings, sequence-based synteny in some regions of the genome will not be as strong as hoped.

Comparing both the soybean BACs with the *Medicago* and *Arabidopsis* sequences shows that synteny in this region is not straightforward, rather there is a network of synteny identified by fragmented blocks of genes. For example, gene 20 is in common between *Arabidopsis* and *Medicago* and only one homeologue of soybean. Gene 17 is in common between both homeologues of soybean and *Medicago*, but not *Arabidopsis*, whereas gene 19 is common between *Arabidopsis* and *Medicago* but is not found on either homeologues of soybean. Taken together, the information from these syntenic regions provides a consolidated view (Langham et al. 2004) of the relationships between these species. Given the fragmented sequence of the *Medicago* genome and the potential for more homeologues in soybean, the reconstruction of an ancestral genome sequence is not possible.

Tandemly Duplicated HCBT Genes

When identifying syntenic blocks in *Medicago* and *Arabidopsis*, tandemly amplified arrays of HCBT genes were identified in all 3 genomes (Figure 1). In *Medicago*, only 3 copies of HCBT were identified and clustered sequentially on 1 BAC, AC122728. In *Arabidopsis*, 3 clusters of HCBT genes could be identified; 2 clusters on chromosome 5, 1 with 3 copies and the other with 2; and 1 cluster of 3 genes on chromosome 3 (Figure 1). It should be noted that almost none of the surrounding genes in either *Medicago* or *Arabidopsis* showed any synteny to the soybean sequences. One BAC, AP007820, did identify a single HCBT gene in

Lotus with an *e* value of 0; however, this BAC was still only Phase I with 25 unordered pieces.

Conserved tandem duplications of HCBT genes in all 3 species suggest that there is a biologically relevant function for these clusters. HCBT functions as the first step in phytoalexin biosynthesis (Yang et al. 1997) and as such may have a role in pathogen response. Tandem arrays of resistance genes are thought to provide a broader range of response to pathogens (Graham et al. 2002). These HCBT clusters found in all these species raise the question as to when the clusters were formed, in other words, if these tandem duplications occurred prior to or postspecies divergence. Alternatively, these duplications may have occurred prior to divergence, and concerted evolution by gene conversion has homogenized each gene family such that sequence similarity suggests independent duplications.

A parsimony-based phylogenetic tree with these clustered HCBT sequences suggests that the tandem duplication of these genes likely occurred after speciation; in other words, each cluster of tandemly arrayed genes was independently formed. We found that genes from one species always form single clades, indicative of independent duplication events (Figure 2). The independent formation of these gene clusters supports the proposed role of these genes in pathogen response (Schlueter et al. 2006).

Although previous studies have suggested that hypersynteny may exist between the legumes (Mudge et al. 2005), only a limited network of synteny could be identified between soybean, *Medicago*, and *Arabidopsis*. Further studies of homeologous regions in soybean are warranted to further understand the evolutionary history of paleopolyploid genomes. The forthcoming whole-genome sequence of soybean (Jackson et al. 2006) will permit us to answer in detail the remaining questions about macro- and microsynteny among these species.

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