Development and bin mapping of gene-associated interspecific SNPs for cotton (*Gossypium hirsutum* L.) introgression breeding efforts

Amanda M Hulse-Kemp¹,², Hamid Ashrafi³, Xiuting Zheng¹, Fei Wang¹, Kevin A Hoegenauer¹,², Andrea BV Maeda¹, S Samuel Yang¹,⁴, Kevin Stoffel³, Marta Matvienko³,⁵, Kimberly Clemons⁶, Joshua A Udall⁶, Allen Van Deynze³, Don C Jones⁷ and David M Stelly¹,²*

**Abstract**

**Background:** Cotton (*Gossypium spp.*) is the largest producer of natural fibers for textile and is an important crop worldwide. Crop production is comprised primarily of *G. hirsutum* L., an allotetraploid. However, elite cultivars express very small amounts of variation due to the species monophyletic origin, domestication and further bottlenecks due to selection. Conversely, wild cotton species harbor extensive genetic diversity of prospective utility to improve many beneficial agronomic traits, fiber characteristics, and resistance to disease and drought. Introgression of traits from wild species can provide a natural way to incorporate advantageous traits through breeding to generate higher-producing cotton cultivars and more sustainable production systems. Interspecific introgression efforts by conventional methods are very time-consuming and costly, but can be expedited using marker-assisted selection.

**Results:** Using transcriptome sequencing we have developed the first gene-associated single nucleotide polymorphism (SNP) markers for wild cotton species *G. tomentosum*, *G. mustelinum*, *G. armourianum* and *G. longicalyx*. Markers were also developed for a secondary cultivated species *G. barbadense* cv. 3–79. A total of 62,832 non-redundant SNP markers were developed from the five wild species which can be utilized for interspecific germplasm introgression into cultivated *G. hirsutum* and are directly associated with genes. Over 500 of the *G. barbadense* markers have been validated by whole-genome radiation hybrid mapping. Overall 1,060 SNPs from the five different species have been screened and shown to produce acceptable genotyping assays.

**Conclusions:** This large set of 62,832 SNPs relative to cultivated *G. hirsutum* will allow for the first high-density mapping of genes from five wild species that affect traits of interest, including beneficial agronomic and fiber characteristics. Upon mapping, the markers can be utilized for marker-assisted introgression of new germplasm into cultivated cotton and in subsequent breeding of agronomically adapted types, including cultivar development.

**Keywords:** Cotton, *Gossypium barbadense*, *Gossypium tomentosum*, *Gossypium mustelinum*, *Gossypium armourianum*, *Gossypium longicalyx*, RNA-seq, Interspecific SNP

* Correspondence: stelly@tamu.edu
¹Department of Soil and Crop Sciences, Texas A&M University, College Station, Texas, USA
²Genetics Graduate Program, Texas A&M University, College Station, Texas, USA
Full list of author information is available at the end of the article

© 2014 Hulse-Kemp et al.; licensee BioMed Central Ltd. This is an Open Access article distributed under the terms of the Creative Commons Attribution License (http://creativecommons.org/licenses/by/4.0), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly credited. The Creative Commons Public Domain Dedication waiver (http://creativecommons.org/publicdomain/zero/1.0/) applies to the data made available in this article, unless otherwise stated.
Background

Cotton (Gossypium *spp.*) is the leading natural fiber crop worldwide and an important contributor to the economies of nearly 100 countries. The genus *Gossypium* is also an important model species for polyploidy and the biological processes of cell wall elongation and cellulose biosynthesis in fiber cells. This clade consists of approximately 45 diploid species and five allotetraploid species. Genomes of the allotetraploid species have 52 chromosomes (2*n* = 4*x* = 52) and are believed to have originated from a single polyploidization event between an A-genome diploid (n = 2*x* = 26) and a D-genome diploid (n = 2*x* = 26) approximately 1–2 million years ago [1]. The five allotetraploid species share a basic AD genome architecture. Chromosomes of the *G. hirsutum* genome ([AD]_1) have been numbered according to their evolutionary origins and meiotic pairing relationships. Chromosomes 1–13 comprise the “A” sub-genome (A_1) that originated from the extinct A-genome diploid ancestor and chromosomes 14–26 comprise the “D” sub-genome (D_1) that originated from the extinct D-genome diploid ancestor. There are four major cultivated species worldwide, two diploids *G. arboreum* (A_2 genome) and *G. herbaceum* (A_1) and two allotetraploids *G. hirsutum* L. or Upland cotton and *G. barbadense* L. ([AD]_2), extra long-staple Pima, Egyptian cotton or Sea Island cotton. Upland cotton cultivation represents over 95% of the fiber produced worldwide due to its high yield, but generally Pima cotton the next most cultivated cotton, exhibits longer, stronger, and finer fiber. Upland cotton has a very narrow genetic base due to multiple bottleneck events including, polyploidization, domestication and continuous selection. It has been suggested and experimentally tested that current Upland cultivars descend from only about a dozen introgressions and therefore exhibit an extremely small amount of diversity [2,3]. With such small diversity in elite cotton germplasm, it is unlikely that sufficient variation for agronomically important traits, such as, fiber properties, yield, disease and insect resistance, drought tolerance and changing atmospheric conditions will be found within currently available elite breeding germplasm. Wild cotton species harbor large numbers of unique genes, which upon introgression may provide novel diversity for genetic improvement.

Diploid cotton species have been shown to have many disease and insect resistance traits, as well as improved fiber characteristics. The diploid *G. longicalyx* Hutch and Lee (F_1) is the only member of the F-genome clade and is native to Africa. It has been shown to have resistance to pathogens, such as reniform nematode [4], and to have beneficial genes for fiber quality [5]. The diploid *G. armstrongianum* Kearney (D_21) belongs to the D-genome clade and is a wild species found in Mexico. It has been shown to exhibit resistance to the whitefly [6], which is the vector for many cotton pathogens such as the leaf curl virus [7]. The diploid species exhibit a large range of relative genome sizes. Due to the difference in chromosome number between diploids and cultivated cotton, methods to move genes from diploids into cultivated tetraploid cotton using synthetic tri-species hybrids have been devised to introgress desired diploid segments through breeding [8,9].

While crossing cultivated tetraploid cotton directly with diploid species is difficult, allotetraploid species can be easily interbred and then backcrossed to move desired segments into cultivated material. The tetraploid *G. tomentosum* Nuttal ex Seeman originates from the Hawaiian islands and produces a small amount of short, reddish brown fiber. *G. tomentosum* has been found to show resistance against the cotton leaf hopper, *Amrasca biguttula biguttula*, and thrips, *Frankliniella occidentalis* [6]. The tetraploid *G. mustelinum* Meers ex Watt is from Brazil and also produces a small amount of lint. Using HPLC analysis, *G. mustelinum* has been shown to have the highest leaf concentrations of terpenoid aldehydes that affect insect resistance [10]. *G. barbadense* originates from South America and is a cultivated species which represents about five percent of the annual worldwide fiber crop. This tetraploid exhibits excellent fiber quality characteristics for fiber length, micronaire and high strength relative to *G. hirsutum*.

Many of the mapping efforts in cotton have consisted of interspecific biparental populations of *G. hirsutum* × *G. barbadense* which offers a higher polymorphism rate than intraspecific crosses, and segregation for superior fiber quality characteristics. Moderate density linkage maps have been created using restriction fragment length polymorphisms (RFLPs) [11,12], amplified fragment length polymorphisms (AFLPs) [13] and simple sequence repeats (SSRs) [14,15]. SSRs have also been used for wide-cross whole-genome radiation hybrid (WWRH) mapping for production of syntenic groups [16,17]. A consensus map was recently created which integrated all of the previous mapping efforts [18]. While single nucleotide polymorphisms (SNPs) represent the most prevalent category of polymorphisms available within the genome, few studies have developed and mapped SNPs in cotton [14,19]. SNP development efforts to-date have produced relatively few numbers of SNPs using different genome reduction methods in cultivated species [19–23].

An aspect of polyploid genomes that creates difficulties during SNP development is that there are two indistinguishable types of SNPs in polyploid sequence data: homeologous sequence variants or “homeo-SNPs” and traditional SNPs or “allele-SNPs” [24]. A catalogue of homeo-SNPs, which are differences between the A-genome and D-genome diploid species, was recently identified in *Gossypium* diploid and tetraploid genomes.
[25,26]. In cotton tetraploids, five million homeo-SNPs were found between the \( A_T \) and \( D_T \) subgenomes, which was facilitated by recent publication of the reference genome sequence for \( G. raimondii \) (\( D_5 \)) [27]. The \( D_5 \) genome is regarded as the closest living diploid relative to the \( D \)-genome ancestor of current AD-allotetraploid species [28]. It has been hypothesized that the catalogued homeo-SNPs can possibly be used to filter putative SNPs when sequence reads are aligned within the framework of the base-pair coordinates of reference diploid genomes [27,29]. While homeo-SNPs may allow for separation of homeologous sequences, they are not directly applicable to breeding. As allele-SNPs identify polymorphisms within a haplotype, experimental assays can be developed to genotype individuals and track favourable and unfavourable alleles. Upon germplasm introgression from wild species, whether diploid or tetraploid, orthologous sequence variants become allele-SNPs. High-density interspecific allele-SNPs distributed across both sets of \( G. hirsutum \) chromosomes will be useful for breeders to efficiently introgress quantitative trait loci (QTLs) and track alleles in marker-assisted selection (MAS) of beneficial traits from donor species.

Traditionally, interspecific introgression breeding efforts are extremely time-consuming and require large amounts of effort and funds. Interspecific genetic introgression into \( G. hirsutum \) has thus far been constrained by the paucity of high-throughput genome-wide markers that would facilitate tracking of introgressed segments. The relative scarcity of SNPs in cultivated allotetraploid cotton reflects the difficulty of developing SNPs for its complex genome, comprised of large repetitive regions and homeologous content due to recent polyploidization. Here, we report a method utilizing the genomic reduction method of transcriptome sequencing to derive interspecific gene-associated SNPs between the genetic standard \( G. hirsutum \) TM-1, and five other species, including the genetic standard \( G. barbadense \) doubled haploid line 3–79, two allotetraploids \( G. tomentosum \) and \( G. mustelinum \), and two diploids \( G. armourianum \) and \( G. longicalyx \). These SNPs will be extremely beneficial for high-density interspecific mapping and will help revolutionize introgression breeding efforts by facilitating MAS-based introgression, genetic dissection, and gene utilization in cultivated cotton.

### Results

#### SNP development

Utilizing the \( G. hirsutum \) (line TM-1) transcriptome assembly produced by Ashrafi et al. (in preparation) consisting of 72,450 contigs covering over 70 M bp with N50 of 1,100 bp, transcriptome sequence reads (Table 1) were aligned and utilized to identity and filter a total of 10,888 SNPs in silico for \( G. barbadense \) line 3–79 relative to \( G. hirsutum \) line TM-1. With the same bioinformatic pipeline, SNPs were also developed for \( G. tomentosum \) (9,520), \( G. mustelinum \) (10,988), \( G. armourianum \) (26,974), and \( G. longicalyx \) (38,217). Reads which mapped to multiple locations were randomly assigned to a single location to achieve higher mapping coverage with limited number of reads. Filtering included removal of theoretical homeo-SNP positions based on an index created by mapping back Illumina TM-1 reads to the assembly. All of the markers identified within a given species were classified according to surrounding polymorphisms for the same species. Marker classifications were based only on species-specific polymorphism data and determined independently for each species. “Class I” was a SNP in which no additional polymorphism was found to exist in the same contig. “Class II” was a SNP in which (an) additional polymorphism(s) was found within the same contig, but the additional polymorphism was outside of 50 base pairs (bp) of the marker. “Class III” was a SNP in which additional polymorphisms were found in the same contig and within 50 bp of the marker. The SNPs for \( G. barbadense \), \( G. tomentosum \), \( G. mustelinum \), \( G. armourianum \) and \( G. longicalyx \) were classified according to these criteria (Table 2).

#### Removal of redundant markers

SNPs that were redundant across species were reduced to a single instance by means of progressive comparisons (see Methods). The overlap with intraspecific \( G. hirsutum \) markers (Ashrafi et al. - in preparation) was low, e.g., only 3.3% or 367 markers of the \( G. hirsutum \) line TM-1 reads to the assembly. All of the markers identified within a given species were classified according to surrounding polymorphisms for the same species. Marker classifications were based only on species-specific polymorphism data and determined independently for each species. “Class I” was a SNP in which no additional polymorphism was found to exist in the same contig. “Class II” was a SNP in which (an) additional polymorphism(s) was found within the same contig, but the additional polymorphism was outside of 50 base pairs (bp) of the marker. “Class III” was a SNP in which additional polymorphisms were found in the same contig and within 50 bp of the marker. The SNPs for \( G. barbadense \), \( G. tomentosum \), \( G. mustelinum \), \( G. armourianum \) and \( G. longicalyx \) were classified according to these criteria (Table 2).

### Table 1 Transcriptome sequence information

| Species           | Sample | Raw reads (#) | Trimmed reads (#) | Mapped reads (#) | Depth | Reference coverage (%) |
|-------------------|--------|---------------|-------------------|------------------|-------|------------------------|
| \( G. barbadense \) | 3-79   | 101,276,621   | 101,276,621       | 43,519,596       | 48.45 | 84%                    |
| \( G. tomentosum \) | 19909036.05 | 63,119,599   | 63,118,203        | 38,439,408       | 31.77 | 87%                    |
| \( G. mustelinum \) | 200508123.02 | 65,940,564   | 65,940,111        | 40,767,777       | 33.07 | 86%                    |
| \( G. armourianum \) | D2-1-6 | 53,279,426   | 53,279,087        | 20,266,019       | 20.90 | 68%                    |
| \( G. longicalyx \) | 200908137.04 | 52,050,537   | 52,050,305        | 23,393,277       | 24.50 | 65%                    |

Raw and processed read information of Illumina GA-II (Solexa) sequence generated from RNA-Seq libraries for \( G. barbadense \), \( G. tomentosum \), \( G. mustelinum \), \( G. armourianum \), and \( G. longicalyx \).
redundant *G. barbadense* SNPs were identified, 2,647 were Class I, 5,660 were Class II (Additional file 1), and 1,189 were Class III (Additional file 2). In addition, when the *G. barbadense* set was BLASTed against itself, 1,025 markers were redundant within the *G. barbadense* set (Additional file 3). SNPs of this redundant nature have been identified and listed separately for each species, so that they can be avoided (or targeted) for future species-specific studies on alternative splicing or gene family composition. For *G. tomentosum* 6,396 SNPs were retained, 811 in Class I, 3,885 in Class II (Additional file 4), and 1,107 in Class III (Additional file 2), while 593 redundant SNPs were listed separately (Additional file 5). A total of 6,663 SNPs were retained for *G. mustelinum*, 822 in Class I, 4,085 in Class II (Additional file 6), 1,107 in Class III (Additional file 2) and 592 redundant SNPs (Additional file 7). For *G. armourianum*, 5,723 Class I, 12,033 Class II (Additional file 8), 4,648 Class III (Additional file 9) and 2,425 redundant SNPs (Additional file 9) were obtained for a total of 24,829 SNPs. Lastly for *G. longicalyx* a total of 34,550 SNPs were identified, including 11,435 in Class I, 15,454 in Class II (Additional file 10), 4,309 in Class III (Additional file 2) and 3,352 SNPs which were redundant and listed separately (Additional file 11). In the final set of 62,555 non-redundant Class I and Class II SNPs for all of the five species, the transition to transversion ratio was 1.63 (38,763/23,792). Non-redundant SNPs were unique in the final set for the SNP and 50bp flanking sequences (now reference as SNP markers).

Alignment of markers to *D*$_5$-reference genome
A moderate share (75.87%) or 47,672 SNP markers in the final set could be aligned to the *Gossypium raimondii* (D$_5$) diploid reference genome sequence. *G. armourianum* had the highest percentage of mapped markers, followed by *G. longicalyx*, and the three tetraploids *G. barbadense*, *G. tomentosum*, and *G. mustelinum*. Nearly all of the mapped markers (99.7%) aligned to one of the thirteen pseudo-chromosome scaffolds, and only 154 (0.3%) markers were aligned to unplaced scaffolds. A bimodal distribution across each *D*$_5$-chromosome was observed when average density of markers was plotted (Figure 2).

Validation of SNPs
Random sets of markers from the non-redundant final set of Class I and Class II SNPs were tested using KASP

---

**Table 2 List of unfiltered SNPs determined for all species**

| Species               | Class I | Class II | Class III | Total   |
|-----------------------|---------|----------|-----------|---------|
| *G. barbadense*       | 3,257   | 6,385    | 1,246     | 10,888  |
| *G. tomentosum*       | 1,520   | 6,526    | 1,474     | 9,520   |
| *G. mustelinum*       | 1,678   | 7,584    | 1,726     | 10,988  |
| *G. armourianum*      | 7,331   | 14,523   | 4,648     | 26,502  |
| *G. longicalyx*       | 14,546  | 18,960   | 11,790    | 45,296  |

Number of SNPs derived in silico relative to *G. hirsutum* inbred line TM-1 for species *G. barbadense*, *G. tomentosum*, *G. mustelinum*, *G. armourianum*, and *G. longicalyx*. SNPs are classified into three categories, Class I are SNPs from contigs with no other SNP residing within the contig, Class II are SNPs from contigs that contain one or more additional SNP outside of the 50-bp flanking sequences (none within), Class III are SNPs from contigs that contain one or more additional SNPs within the 50-bp flanking sequences.

---

**Figure 1 Overlap of SNPs among species.** The overlap and specificity of the Class I and Class II SNPs for *G. barbadense* cv. 3–79, *G. tomentosum*, *G. mustelinum*, *G. armourianum*, and *G. longicalyx*.
end-point assays (LGC Genomics, Beverly, MA, USA) from each species. As random sets of markers for each species were selected for screening prior to development of the final non-redundant set, some markers may have been tested on species other than the ones with which they were associated in the non-redundant list of SNPs (Additional files 2, 3, 4, 5, 6, 7, 8, 9, 10 and 11). This is due to the fact that some markers detected the same SNP between *G. hirsutum* and multiple species and was retained only once in the non-redundant list. The validation status of each marker for tested species is noted in columns B and C of Additional files 2, 3, 4, 5, 6, 7, 8, 9, 10 and 11.

In the final non-redundant data sets, a total of 665 randomly selected markers were tested from the *G. barbadense* Class I and Class II set. Of these, 262 markers were tested on the "*G. barbadense* screening panel" (Figure 3) and 209 (79.8%) had clean clusters which allowed for scoring P1, P2 and F1 genotypes (successful markers). The remaining 403 markers were screened on panels from the other species (*G. tomentosum, G. mustelinum, G. armourianum* or *G. longicalyx*) and 286 (71%) of those were validated to have scoreable genotypes. Sets of markers were also screened which fall under the species-specific data sets, 466 were tested from the *G. tomentosum* set of which 252 were tested on the *G. tomentosum* screening panel which produced 168 (66.7%) successful markers. The other 214 markers were tested on other species and 141 (65.9%) were validated. A total of 138 were tested from the *G. mustelinum* data set, of which 90 were run on the *G. mustelinum* screening panel and 48 were run on other species screening panels. These tests resulted in 61 (67.8%) successful markers from the same-species tests and 27 (56.3%) successful markers from the other-species tests. For the *G. armourianum* data set, 214 markers were tested, of which only 5 were tested on species-specific panels. Overall, 146 out of the 209 (69.9%) markers tested on *G. armourianum* produced successful assays. A small set of 27 markers was tested from the *G. longicalyx* data set, of which 19 (70.4%) generated successful assays. A similar proportion of successful assays was
obtained from SNPs generated for *G. longicalyx* using a different *G. hirsutum* assembly version that was abandoned because it yielded poor results when used to define SNPs in other species. Unique SNPs from this set were extracted and included in Additional file 12.

**Validation of *G. barbadense* SNPs – Wide-cross whole-genome radiation hybrid mapping**

A total of 509 markers were WWRH mapped using 131 WWRH individuals (*G. hirsutum* line TM-1 × irradiated pollen of *G. barbadense* cv. 3–79 [16]). Those markers (124) which were found using a previous version of the bioinformatic pipeline (which produced sets with overall lower success rate) and thus are not in the final set (Additional files 2 and 3) have names and sequences listed in Additional file 13. A total of 60 syntenic groups were produced along with 43 singletons (Additional file 14) that were not integrated into a syntenic group. Most of the groups (52) were anchored onto the *G. raimondii* draft genome sequence (Figure 4) by alignment of markers, as well as by chromosome localization using deficiency mapping with *F*₁ hypo-aneuploids (Figure 5) and/or the presence of marker(s) that were previously linkage-mapped [14]. The markers in Figure 4 are reported in bins because the order of the markers may not be accurately estimated as the WWRH panel did not provide enough power to precisely order markers.

**Deletion analysis of *G. barbadense* SNPs**

The number of WWRH deletions was significantly higher for only 6 (1.2%) of the 509 *G. barbadense* markers used for WWRH mapping, based on Tukey’s boxplot method of outlier detection. These markers were Gb379_011066, Gb379_000467, UCcg10762_238, UCcg10614_274, c4_101926, and c4_44618.

**Functional analysis**

A total of 117 contigs containing 118 SNPs (three of the shared SNPs in Figure 1 are loci which have three alleles so these were not included in the analysis) were found to be shared between all wild species relative to the TM-1 *G. hirsutum* reference. When translation was predicted using AUGUSTUS software (http://bioinf.uni-greifswald.de/augustus/) [30] and *Theobroma cacao* as the model species, 52 out of the 116 translations were found to generate different amino acid sequences or non-synonymous substitutions, which corresponds to a Ka/Ks of 0.8125. As a method of comparison to the first set, a random set of 118 SNPs from the overall non-redundant Class I and II data set was chosen. These SNPs represented 118 different contigs. When the same analysis using AUGUSTUS was performed with this random set of contigs, 33 out of the 109 translations were found to generate different amino acid sequences, Ka/Ks of 0.4342.
Discussion

Interspecific germplasm introgression provides a powerful way of introducing novel beneficial alleles into breeding germplasm of Upland cotton. Its use has been constrained by the long time periods required for introgression and the difficulty of breaking linkage blocks. But when patience is exhibited and recombination has occurred to break linkage blocks to allow for introgression of interspecific segments, highly beneficial products can be obtained. Such is the case with BARBREN which was created to move reniform resistance found in *G. barbadense* into *G. hirsutum* along with superior fiber characteristics [31]. Combining features of *G. barbadense* with *G. hirsutum* has been a long-standing desire, because *G. barbadense* offers many superior fiber trait characteristics but does not produce the high lint yield of traditional *G. hirsutum* cultivars. The large number of *G. barbadense* SNPs identified in this study, 10,521, and particularly the 8,307 Class I and Class II markers will provide markers for a large number of genes in which differences exist between *G. hirsutum* and *G. barbadense*. A modest number, 509 markers, have been validated (from 594 tested) and the majority of these have been anchored to an allotetraploid chromosome by WWRH mapping, therefore relative physical location is known (Figure 4). A larger number of markers and/or larger WWRH panel would have been needed to link all of the singleton markers into the syntenic groups.

![Figure 4 Wide-cross whole-genome radiation hybrid bin map.](https://www.biomedcentral.com/1471-2164/15/945)
Markers within each group were close enough for statistical association, but the number and variety of deletions was insufficient to accurately determine physical order. The average number of plants with a deletion for a given SNP was only 8.9 (6.9%), far short of the optimal deletion rate i.e., 50%. Accurate ordering would require a far larger population of similarly irradiated plants or a population with a much higher deletion rate.

Additional investigation into patterns of deletions showed that only 6 (1.2%) of markers across individuals had a statistically significant number of deletions. This was a statistically insignificant number from the overall set. The relative abundance of these marker deletions suggests that the respective chromosomal segments have higher propensities for deletion and/or post-occurrence recovery of induced deletions. Some chromosomal segments may be more likely to be lost after pollen irradiation, and/or there may be significant differences in selection for/against loss of specific genes/alleles in these segments that correspond to the identified markers.

Placement of the syntenic groups relative to the D5 reference sequence revealed most to be in non-pericentromeric and non-telomeric regions, i.e., similar to the pattern observed for individual markers (Figure 2). SNPs were distributed unevenly across the chromosomes. A bimodal distribution was observed for each pseudo-chromosome scaffold, with large numbers of SNPs near the subtelomeric regions and small numbers in centromeric and telomeric regions, as would be expected given the metacentric nature of cotton chromosomes and the fact that the markers were derived from expressed sequences [32]. In addition all SNPs were found to integrate into a single syntenic group, unlike SSRs which integrate across groups, which implies the majority of SNPs are subgenome specific and will be useful for breeding as identifying a unique position in the genome.

Mapping experiments were focused on *G. barbadense*. Like Upland cotton, it is a cultivated species and represents approximately five percent of the worldwide cotton production. However, *G. barbadense* being not as highly improved as *G. hirsutum*, it retains some alleles that are deleterious when brought into a *G. hirsutum* background. Some research has been done utilizing chromosome substitution lines, in which a single chromosome in the *G. hirsutum* allotetraploid has been replaced with the same allotetraploid chromosome from a different species, for example *G. barbadense* [33]. Many beneficial regions have been identified for yield components as well as fiber quality traits [34,35]. Recombinant inbred lines from these chromosome substitution lines have also been generated recently that will assist in introgression efforts once markers from a high-density dataset such as was developed here have been located for target areas. It has been shown that cryptic beneficial alleles are typically masked in the overall *G. barbadense* background [36].

Like *G. barbadense*, the other wild allotetraploid species, *G. mustelinum* and *G. tomentosum*, included in this study have also been shown to host cryptic beneficial alleles. These species have been integrated into the chromosome substitution line development effort and will provide additional trait resources for movement into a *G. hirsutum* background. Being of allotetraploid genome constitution, most genomic segments from these...
species will easily be moved into *G. hirsutum*. Integrating genes from wild diploid species like *G. longicalyx* and *G. armourianum* is much more complicated, because their diploid genomes are vastly different from the *G. hirsutum* genome. However with the longer divergence time and large number of diploid species available, (~45) compared to uncultivated tetraploid species (~3-5), a much larger number of unique beneficial alleles may be found in diploid *Gossypium* species. Inventive methods of creating synthetic polyploids with diploid species have been devised to facilitate transfer of genetic material into *G. hirsutum*, as was the case for *G. longicalyx*, in order to create Upland cottons with strong resistance to reniform nematodes [37]. Two sister lines with strong reniform nematode resistance, LONREN-1 and LONREN-2, were released but subsequently discovered to suffer early growth season “stunting” suggesting a possible linkage drag or pleiotropic effect [38]. In general, practical utilization of introgressed alien germplasm demands precise genetic manipulation to separate linked beneficial and deleterious alien genes, for which numerous markers are essential. Thus, large numbers of markers are needed for each germplasm source, such as the markers developed here. Class I markers will be exceptionally useful as they can be used for determining haplotype information being the only marker identified within a contig.

Studying shared markers between the different species of varied genome composition relative to cultivated *G. hirsutum* can be used to deduce the theoretical ancestral allele at a locus, as well as to suggest functional properties of a locus. The distribution of shared markers is depicted in Figure 1. For markers at which all wild species share a common allele but *G. hirsutum* differs, it can be inferred that the wild species share the ancestral allele and *G. hirsutum* contains an alternative allele. Such alleles are good candidates for being functionally important to domestication, cultivation or agronomic performance, or in linkage disequilibrium with such genes. The non-synonymous to synonymous rate was found to be much higher in the 118 SNPs shared between species (0.8125) than in 118 randomly selection SNPs from the final set (0.4342). This implies that there is a stronger positive selection upon the SNPs where *G. hirsutum* has an allele which is non-ancestral. This further supports the hypothesis that these loci are likely to be important in beneficial traits in *G. hirsutum*.

Some markers within the same species were identified from multiple scaffolds, but were identical in SNP and flanking sequence (Additional files 3, 5, 7, 9, 11). This is likely due to genes from gene families which exhibit very high levels of sequence similarity. Therefore multiple hits are expected for genes that have expanded in the TM-1 or *G. hirsutum* lineage or are duplicated within a genome (paralogs). Another possible explanation is that these hits relate to contigs that contain different isoforms of the same gene. When SNPs derived from multiple isoforms are mapped physically or by linkage, they will locate to a single locus. SNPs from the former case will occupy multiple locations in which different members of the gene family are found. We classified these SNPs separately as they may represent multiple loci throughout the genome and present another level of difficulty for genotyping. The diploid species were found to have many more SNPs exhibiting within-species redundancy, which are marker sequences that are identical but generated from multiple contigs in the assembly, than the tetraploid species. This result was expected because the tetraploid *G. hirsutum* that was utilized as a reference likely received a copy of each gene from ancestors of the A and D subgenomes during polyploidization. Thus relative to each diploid, the reference would have twice as many copies for each gene (assuming no gene loss or duplication has occurred, or co-assembly) and would lead to derivation of the same SNP sequence from each of the homeologous copies found in *G. hirsutum* when analyzing the diploid species.

Conclusions

Markers associated with functional differences between species are essential for generating a feasible system for germplasm introgression via marker-assisted breeding for beneficial agronomic traits. Future large-scale mapping, fine mapping, and genome-wide association analysis efforts to associate the markers developed here as diagnostic markers for traits of interest will allow for marker assisted selection and back crossing to speed up introgression efforts. Advancements in interspecific germplasm introgression are likely to create opportunities for profound improvement of cotton *G. hirsutum* cultivars and will allow for more sustainable provisioning of society in the face of population growth, evolving pathogens and insects, drought and changing environments.

Methods

Plant materials

The seed of *G. barbadense* L. (AD)2 genetic standard line 3–79, *G. tomentosum* (AD)3 plant number 19909036.05 from the Beasley Lab collection, *G. mustelinum* (AD)4 plant number 200508123.02 from the Beasley Lab collection, *G. armourianum* (D2–1) accession D2-1-6, and *G. longicalyx* (F1) plant number 200908137.04 from the Beasley Lab collection were planted at Texas A&M University. Young leaf tissues were sampled from each plant and used to isolate total RNA using the Qiagen RNeasy Mini Kit per manufacturer instructions. RNA isolates were quantified using NanoDrop spectrophotometry (Thermo Scientific, Wilmington, USA) and checked for quality by gel electrophoresis. PolyA RNA was extracted using double purification with oligo dT
Dynal beads. Illumina RNA-Seq libraries were prepared using the manufacturer protocol (Illumina Inc, San Diego, USA). Libraries were normalized by denaturation and rehybridization in NaCl and TMAC (tetrA-methyl-ammonium-chloride) buffers [39] and then treated with Duplex Specific Nuclease to digest cDNAs from highly abundant transcripts [40]. The treated library was then re-amplified for 12 PCR cycles using Illumina library primers. The libraries were then single-read sequenced using the Illumina Genome Analyzer II for 85 cycles (Table 1). Raw single-read sequence files were uploaded to NCBI under BioProject PRJNA203021 and SRA numbers (SRX457172 – G. barbadense, SRX472724 - G. tomentosum, SRX - 474879/SRR174699 G. mustelinum, SRX474240/SRR1174039 and SRR1174041-G. armourianum, and SRX474242/SRR1174179 and SRR1174182 - G. longicalyx).

Wide-cross whole-genome radiation (WWRH) individuals [16] were planted and maintained at Texas A&M University. Small leaves were collected from each plant and extracted using the Qiagen DNeasy plant extraction kit per manufacturer instructions.

SNP development
Reads from each species were trimmed for quality and then aligned to the G. hirsutum L. assembly created from genetic standard line, TM-1 (GALV00000000.1 Ashrafi et al. - in preparation), using CLC Genomics Workbench (V5.0). Reads which mapped to multiple locations were randomly assigned to a single location. Putative SNPs between TM-1 and each species were identified one accession at a time. The mapping data were exported as BAM files to a Linux server and SAMtools were used to call variants. Subsequent rounds of parameter tweaking resulted in the final pipeline used for SNP development. The resulting pileup files were filtered using the filter pileup Perl script in Galaxy (https://main.g2.bx.psu.edu/) [41] to remove indels and positions with less than coverage of 3. The resulting file was then further filtered using an in-house Perl script which required two genotypes to be homozygous for different bases with minimum coverage of 10. Putative SNPs were then removed from the list if they were located within 50 bases of predicted intron-exon boundary on the TM-1 assembly using SGN (http://solgenomics.net/) intron finder tool. SNPs were further filtered to remove theoretical homeo-SNP positions based on allele-SNP calls generated when Illumina TM-1 reads were mapped back to the TM-1 reference.

9SNPs from each species were classified based on identification of additional SNPs. Class I was defined as SNPs from contigs with no other SNP residing within the contig. Class II was defined as SNPs from contigs that contained one or more additional SNP outside of the 50-bp flanking sequences (none within). Class III was defined as SNPs from contigs that contained one or more additional SNPs within the 50-bp flanking sequences.

Removal of redundant markers
A FASTA file containing all in silico-derived SNPs was used for BLAST analysis (v2.2.27) against a FASTA file containing all G. hirsutum markers from the Ashrafi et al. (in preparation) dataset. Markers were removed from the in silico set if BLAST analysis showed 100% identity over 100% length of the sequence to the G. hirsutum data set. The remaining set was then BLASTed against itself to determine identical markers within the set. Markers with hits in other species groups were removed in a hierarchical method, leaving markers in the highest set, based on the hierarchy G. barbadense, G. tomentosum, G. mustelinum, G. armouianum, and G. longicalyx. Markers with hits within the same species were separated into a data set containing “overlap markers” (Additional files 3, 5, 7, 9, 11). The result was a final non-redundant data set of Class I and Class II markers for each species (Additional files 1, 4, 6, 8, and 10). The final non-redundant set of Class I and Class II SNPs for each species were submitted to NCBI’s dbSNP (ss974702651-ss974710721 for G. barbadense, ss1026506434-ss1026511516 for G. tomentosum, ss1026511517-ss1026516811 for G. mustelinum, ss1026516812-ss1026536246 for G. armouianum, and ss1026536247-ss1026565496 for G. longicalyx). Non-redundant Class III SNPs were compiled into Additional file 2 for all species.

Alignment of markers to D5-reference genome
The non-redundant SNPs and overlap markers were aligned to the G. raimondii (D5) reference genome sequence [27] using Burrows-Wheeler Aligner (BWA) in Galaxy [41] using default settings. Alignment positions were corrected to note the SNP base positions. SNPs were separated into files based on D5 genome scaffold alignment. Scaffold files were sorted by genome position. Densities of markers along the D5 genome scaffolds were plotted using densityPlot in the R program [42] over scaffold position.

SNP validation
Subsets of SNPs from the subsequent rounds of bioinformatic filtering were selected for experimental testing using the LGC KASP assays (Beverley, USA). Assay primers were developed using Batch Primer3 with an optimal primer Tm of 57°C (minimum 55°C, maximum 60°C, maximum difference between primers of 5°C), optimal product size of 50 base pairs (minimum 50 base pairs, maximum 100 base pairs) and the default settings were used for the remaining parameters. Primers were mixed at the dilutions specified by LGC then used to perform KASP
assays on small screening panels containing duplicates, e.g.,
the panel used to screen G. barbadense markers contained
2 G. hirsutum L. TM-1, 2 G. barbadense 3–79, 2 euploid
F1 (G. hirsutum x G. barbadense), 4 RILs from a G. hirsutum
x G. barbadense mapping population, and 2 non-
template (negative) controls (Figure 3). Plates were initially
run for the recommended 38 cycles on the LGC SNP plat-
form, centrifuged then read on the Pherastar plate reader.
The Pherastar files were imported into KlusterCaller soft-
ware for genotyping (Figure 5). If the plates were deter-
mined to be insufficiently clustered, additional sets of
3 cycles were added and the plates were re-read and re-
imported, until scoreable clusters were formed or the
marker was deemed to be unacceptable.

Wide-cross whole-genome radiation hybrid mapping
Those G. barbadense markers which clustered well from
all rounds of development used for parameter tweaking
until the final pipeline was reached were then run on a
“full-panel” containing duplicates of the parental and F1
controls (G. hirsutum line TM-1, G. barbadense cultivar
3–79, euploid F1), G. hirsutum cultivar DP-90, G. barba-
dense cultivar Phytoegen800, 131 wide-cross whole-genome
radiation hybrid individuals, 47 F1 hypo-aneuploid lines,
and 4 non-template negative controls. Genotypes were
manually curated. All questionable genotypes were listed
as unscored. A shift of genotype from the F1 heterozygote
cluster to the homozygous 3–79 cluster was interpreted as
a deletion in the respective interspecific WWRH or hypo-
aneuploid F1 cytogenetic stock (Figure 1). Genotype files
were manipulated to note presence (1) or absence (0) of a
deletion in the WWRH plants.

An additional set of markers from two previous stud-
ies, Van Deynze et al. [20] and Byers et al. [19], were also
genotyped using KASP assays. Genotypes for these
markers were also manipulated to note presence or ab-
sence of a deletion in the WWRH plants.

SNP markers which showed no deletions among the
131 WWRH samples were removed from the WWRH
mapping analysis. Genotype files in binary format were
analyzed using Carthagene, with a LOD score of 3.0 and
mapping distance within 100 cR. From the resulting syn-
tenic groups, the singleton groups were removed and
classified as non-linked markers (Additional file 14).
Those syntenic groups with two or more markers were
subjected to finishing methods using annealing, flips and
polishing to determine the final group order. The result-
ing syntenic groups were cross-referenced with the D5
alignments of individual markers, as well as the chromo-
some locations determined by deletion analysis with the
F1 hypo-aneuploids. Syntenic groups and their relation-
ships based on alignment to the D5 scaffolds were plotted
(Figure 4) using Strudel software [43].

Deletions analysis
The numerical distribution of deletions in the radiation
hybrids per marker was analyzed by a box plot method
to determine the number of deletions that would be sta-
tistically significant. The first and third quartile were de-
termined along with the mean, then the inner quartile
range (IQR) was utilized to calculate the threshold for
outliers greater than 1.5 times the IQR. Markers which
had numbers of deletions beyond the threshold were de-
termined to have a significantly different number of de-
teleions than expected.

Functional analysis
Contigs for which the SNPs represented identical differences
from G. hirsutum TM-1 for all five species from the refer-
ence were parsed into a FASTA file containing 117 contigs
for 118 SNP. The reference FASTA file was modified to con-
tain the alternate SNP allele(s) using an in house Perl script.
Both the reference and alternate FASTA files were analyzed
using AUGUSTUS [30] to predict translation start and end
sites using Theobroma cacao as the model species. Non-
ynonymous and synonymous changes between the refer-
ence and alternate files were calculated. Predicted amino
acid coding sequences were then investigated using
TBLASTX against NCBI’s non-redundant database. BLAST
results were parsed to contain the top hit with evalue cut off
of $e^{-8}$. The differences between predicted protein products
were investigated. The same analysis was performed using a randomly
selected set of 118 SNP from the final overall Class I and Class II data set.

Availability of supporting data
The data sets supporting the results of this article are in-
cluded within the article and its additional files.

Additional files

Additional file 1: G. barbadense cv. 3–79 SNPs relative to G.
hirsutum line TM-1. SNPs and 50-bp flanking sequences for in silico
derived SNPs in G. barbadense cv. 3–79 relative to G. hirsutum line TM-1.
SNPs have been classified into two categories, Class I are SNPs from
contigs with no other SNP residing within the contig. Class II are SNPs
from contigs that contain one or more additional SNP outside of the
50-bp flanking sequences (none within). Columns 4 and 5: Alignment
position and scaffold information to G. raimondii (D5) draft genome
sequence using BWA. Column 6 indicates overlap information for
G. barbadense markers with G. tomentosum, G. mustelum, G. armourianum,
and G. longicalyx, where the Arabic numeral indicates number of shared species
and subsequent abbreviations indicate with which of the above species the
marker is shared (Gt, Gm, Ga and Gl, respectively).

Additional file 2: Five species Class III SNPs. Class III SNPs for
G. barbadense cv. 3–79, G. tomentosum, G. mustelum, G. armourianum,
and G. longicalyx. Class III are SNPs from contigs that contain one or more
additional SNPs within the 50-bp flanking sequences. Class III SNP sequences
are provided for reference, but have been shown to have issues during
experimental screening using KASP assays.

Additional file 3: G. barbadense cv. 3–79 SNPs relative to G. hirsutum
line TM-1 within-species overlapping markers. SNPs and 50-bp flanking
sequences for in silico-derived SNPs of G. barbadense (cultivar 3-79) relative to G. hirsutum (genetic standard line TM-1) that have been found to be identical for SNP and flanking sequence within the G. barbadense data set. SNPs have been classified into two categories, Class I are SNPs from contigs with no other SNP residing within the contig. Class II are SNPs from contigs that contain one or more additional SNP outside of the 50-bp flanking sequences (none within). Alignment position and scaffold information to G. raimondii (Dj.) draft genome sequence using BWA. Column 6 indicates overlap information for G. tomentosum markers with G. mustelimum, G. armourianum, and G. longicalyx, where the Arabic numeral indicates number of shared species and subsequent abbreviations indicate with which of the above species the marker is shared (Ga, Gt, Gm, Ga and Gl, respectively).

**Additional file 4:** G. tomentosum SNPs relative to G. hirsutum line TM-1. SNPs and 50-bp flanking sequences for in silico-derived SNPs of G. tomentosum relative to G. hirsutum line TM-1. SNPs have been classified into two categories, Class I are SNPs from contigs with no other SNP residing within the contig. Class II are SNPs from contigs that contain one or more additional SNP outside of the 50-bp flanking sequences (none within). Alignment position and scaffold information to G. raimondii (Dj.) draft genome sequence using BWA. Column 6 indicates overlap information for G. tomentosum markers with G. mustelimum, G. armourianum, and G. longicalyx, where the Arabic numeral indicates number of shared species and subsequent abbreviations indicate with which of the above species the marker is shared (Ga, Gt, Gm, Ga and Gl, respectively).

**Additional file 5:** G. tomentosum SNPs relative to G. hirsutum line TM-1 within species overlapping markers. SNPs and 50-bp flanking sequences for in silico-derived SNPs of G. tomentosum relative to G. hirsutum line TM-1 that have been found to be identical for SNP and flanking sequence within the G. tomentosum data set. SNPs have been classified into two categories, Class I are SNPs from contigs with no other SNP residing within the contig. Class II are SNPs from contigs that contain one or more additional SNP outside of the 50-bp flanking sequences (none within). Alignment position and scaffold information to G. raimondii (Dj.) draft genome sequence using BWA. Column 6 indicates overlap information for G. tomentosum markers with G. mustelimum, G. armourianum, and G. longicalyx, where the Arabic numeral indicates number of shared species and subsequent abbreviations indicate with which of the above species the marker is shared (Ga, Gt, Gm, Ga and Gl, respectively).

**Additional file 6:** Gossypium mustelimum SNPs relative to G. hirsutum line TM-1. SNPs and 50-bp flanking sequences for in silico-derived SNPs of G. mustelimum relative to G. hirsutum line TM-1. SNPs have been classified into two categories, Class I are SNPs from contigs with no other SNP residing within the contig. Class II are SNPs from contigs that contain one or more additional SNP outside of the 50-bp flanking sequences (none within). Alignment position and scaffold information to G. raimondii (Dj.) draft genome sequence using BWA. Column 6 indicates overlap information for G. mustelimum markers with G. armourianum, and G. longicalyx, where the Arabic numeral indicates number of shared species and subsequent abbreviations indicate with which of the above species the marker is shared (Ga, Gt, Gm, Ga and Gl, respectively).

**Additional file 7:** G. mustelimum SNPs relative to G. hirsutum line TM-1 within species overlapping markers. SNPs and 50-bp flanking sequences for in silico-derived SNPs of G. mustelimum relative to G. hirsutum line TM-1 that have been found to be identical for SNP and flanking sequence within the G. mustelimum data set. SNPs have been classified into two categories, Class I are SNPs from contigs with no other SNP residing within the contig. Class II are SNPs from contigs that contain one or more additional SNP outside of the 50-bp flanking sequences (none within). Alignment position and scaffold information to G. raimondii (Dj.) draft genome sequence using BWA. Column 6 indicates overlap information for G. mustelimum markers with G. armourianum, and G. longicalyx, where the Arabic numeral indicates number of shared species and subsequent abbreviations indicate with which of the above species the marker is shared (Ga, Gt, Gm, Ga and Gl, respectively).

**Additional file 8:** G. armourianum SNPs relative to G. hirsutum line TM-1. SNPs and 50-bp flanking sequences for in silico-derived SNPs of G. armourianum relative to G. hirsutum line TM-1. SNPs have been classified into two categories, Class I are SNPs from contigs with no other SNP residing within the contig. Class II are SNPs from contigs that contain one or more additional SNP outside of the 50-bp flanking sequences (none within). Alignment position and scaffold information to G. raimondii (Dj.) draft genome sequence using BWA. Column 6 indicates overlap between the two species G. armourianum markers with G. longicalyx.

**Additional file 9:** G. armourianum SNPs relative to G. hirsutum line TM-1 within species overlapping markers. SNPs and 50-bp flanking sequences for in silico-derived SNPs of G. armourianum relative to G. hirsutum line TM-1 that have been found to be identical for SNP and flanking sequence within the G. armourianum data set. SNPs have been classified into two categories, Class I are SNPs from contigs with no other SNP residing within the contig. Class II are SNPs from contigs that contain one or more additional SNP outside of the 50-bp flanking sequences (none within). Alignment position and scaffold information to G. raimondii (Dj.) draft genome sequence using BWA. Column 6 indicates overlap between the two species G. armourianum markers with G. longicalyx.

**Additional file 10:** G. longicalyx SNPs relative to G. hirsutum line TM-1. SNPs and 50-bp flanking sequences for in silico-derived SNPs of G. longicalyx relative to G. hirsutum line TM-1. SNPs have been classified into two categories, Class I are SNPs from contigs with no other SNP residing within the contig. Class II are SNPs from contigs that contain one or more additional SNP outside of the 50-bp flanking sequences (none within). Alignment position and scaffold information to G. raimondii (Dj.) draft genome sequence using BWA.

**Additional file 11:** G. longicalyx SNPs relative to G. hirsutum line TM-1 within species overlapping markers. SNPs and 50-bp flanking sequences for in silico-derived SNPs of G. longicalyx relative to G. hirsutum line TM-1. SNPs have been classified into two categories, Class I are SNPs from contigs with no other SNP residing within the contig. Class II are SNPs from contigs that contain one or more additional SNP outside of the 50-bp flanking sequences (none within). Alignment position and scaffold information to G. raimondii (Dj.) draft genome sequence using BWA.

**Additional file 12:** Additional G. longicalyx SNPs (Class I, Class II & Class III). “Specific” G. longicalyx SNP markers, which were determined using an alternative version of the TM-1 assembly. Markers are classified into three categories, Class I are SNPs from contigs with no other SNP residing within the contig. Class II are SNPs from contigs that contain one or more additional SNP outside of the 50-bp flanking sequences (none within). Class III are SNPs from contigs that contain one or more additional SNPs within the 50-bp flanking sequences.

**Additional file 13:** G. barbadense SNPs relative to G. hirsutum line TM-1 markers from different pipelines included in wide-cross whole-genome radiation hybrid mapping. SNPs and 50-bp flanking sequence for in silico derived SNPs in G. barbadense relative to G. hirsutum (genetic standard line TM-1). SNP and flanking sequence are listed for markers which did not overlap the set of SNPs produced using the final bioinformatic analyses to produce markers in Additional files 1 and 3.

**Additional file 14:** Singleton markers from G. barbadense whole-genome radiation hybrid mapping. List of markers which did not fall into any syntenic group from Carthage analysis with G. barbadense markers used for whole-genome radiation hybrid mapping.

**Abbreviations**

SNP: Single nucleotide polymorphism; RFLP: Restriction fragment length polymorphism; AFLP: Amplified fragment length polymorphism; SSR: Simple sequence length repeat; WWRH: Wide-cross whole-genome radiation hybrid; QTL: Quantitative trait loci; MAS: Marker assisted selection.

**Competing interests**

The authors declare that they have no competing interests.

**Authors’ contributions**

AHK, HA, AVD, and DS designed and managed various aspects of the project. MW and KS constructed normalized RNA-Seq libraries and assisted with data analysis. SY designed the initial RNAseq SNP development. AHK, HA, AVD, and DS designed and managed various aspects of the project. FW, XZ, and ABVM screened GT, GM, GA and GL markers. KC.

**Hulse-Kemp et al. BMC Genomics 2014, 15:945**

http://www.biomedcentral.com/1471-2164/15:945

Page 12 of 14
and JAU genotyped markers from Byers et al. 2011. AHK analyzed the data and drafted the manuscript. DCJ contributed to project development and community involvement. All authors have read and approved the final manuscript.

Acknowledgements

We thank the Whole Systems Genomics Initiative (WSGI) at Texas A&M University for providing computation resources and systems administration support for the WSGI HPC Cluster. The authors thank Steve Todd for development of the RH panel. This work was supported by funding from Cotton Incorporated grant No. 05-671 (WWMH), 08-386 and 13-694 (SNPs) and the National Science Foundation, Plant Genome Research Program (NSF-PGRP) grant No. IOS1025947.

Author details

1. Department of Soil and Crop Sciences, Texas A&M University, College Station, Texas, USA. 2. Genetics Graduate Program, Texas A&M University, College Station, Texas, USA. 3. Seed Biotechnology Center, University of California, Davis, California, USA. 4. Monsanto Company, Molecular Breeding Technology, 700 Chesterfield Parkway West, C224-B, Chesterfield, MO, 63017, USA. 5. Current Address: CLC Bio, a QIAGEN Company, Davis, CA, USA. 6. Plant and Wildlife Science Department, Brigham Young University, 2951 WSB, Provo, UT, USA. 7. Cotton Incorporated, Cary, NC, USA.

Received: 12 July 2014 Accepted: 3 October 2014
Published: 30 October 2014

References

1. Wendel JF, Brubaker C, Alvarez I, Cronn R, Stewart JM: Evolution and Natural History of the Cotton Genus. In Genetics and Genomics of Cotton. Volume 3. Edited by Paterson AH. New York: Springer; 2009:3–22.

2. Richmond T: Procedures and methods of cotton breeding with special reference to American cultivated species. Adv. Genet. 1950, 4:213–245.

3. Van Esbroeck G, Bowman DT: Resistant germplasm in related plants to Gossypium species and other cultivated species. J Cotton Sci 2013, 17:151–159.

4. Robinson A, Bell A, Dighe N, Menz M, Nichols R, Stelly DM: Introgressions interspécifiques chez le cotonnier. Edited by Ignacimuthu S, Jayaraj S. New Delhi: Alpha Science Int Ltd; 2005.

5. Wendel JF, Brubaker C, Alvarez I, Cronn R, Stewart JM, Gutierrez O, Hayes R, Stelly DM: A novel hybrid gene prediction method employing protein multiple sequence alignments. Bioinformatics 2011, 27:757–763.

6. Byers RL, Harker DB, Yourstone SM, Uddal JA: Development and mapping of SNP assays in allotetraploid cotton. Theor Appl Genet 2012, 124:1201–1214.

7. Altif M, Stewart J, Murphy J: Survey of Cotton Germplasm for Terpenoid Aldehydes Important in Host Plant Resistance. J Chem Ecol 2013, 39:913–920.

8. Briddon R, Markham P: Virus Res 2000, 75:612–626.

9. Kramer J, Wang F, Sorrells ME: Crop Sci 2013, 53:913–920.

10. Altif M, Stewart J, Murphy J: Survey of Cotton Germplasm for Terpenoid Aldehydes Important in Host Plant Resistance. J Chem Ecol 2013, 39:913–920.

11. Wendel JF, Brubaker C, Alvarez I, Cronn R, Stewart JM, Gutierrez O, Hayes R, Stelly DM: A novel hybrid gene prediction method employing protein multiple sequence alignments. Bioinformatics 2011, 27:757–763.

12. Byers RL, Harker DB, Yourstone SM, Uddal JA: Development and mapping of SNP assays in allotetraploid cotton. Theor Appl Genet 2012, 124:1201–1214.

13. Altif M, Stewart J, Murphy J: Survey of Cotton Germplasm for Terpenoid Aldehydes Important in Host Plant Resistance. J Chem Ecol 2013, 39:913–920.

14. Altif M, Stewart J, Murphy J: Survey of Cotton Germplasm for Terpenoid Aldehydes Important in Host Plant Resistance. J Chem Ecol 2013, 39:913–920.
35. Jenkins JN, Wu J, McCarty JC, Saha S, Gutiérrez O, Hayes R, Stelly DM: Genetic effects of thirteen Gossypium barbadense L. chromosome substitution lines in topcrosses with Upland cotton cultivars: I. Yield and yield components. Crop Sci 2006, 46:1169–1178.

36. Saha S, Wu J, Jenkins J, McCarty J, Stelly D: Interspecific chromosomal effects on agronomic traits in Gossypium hirsutum by AD analysis using intermated G. barbadense chromosome substitution lines. Theor Appl Genet 2013, 126:109–117.

37. Bell AA, Forest Robinson A, Quintana J, Dighe ND, Menz MA, Stelly DM, Zheng X, Jones JE, Overstreet C, Burris E: Registration of LONREN-1 and LONREN-2 germplasm lines of upland cotton resistant to reniform nematode. J Plant Regist 2014, 8:187–190.

38. Zheng X: High-Resolution Recombination to Dissect an Alien Segment of Cotton Chromosome-11 with Resistance to Reniform Nematodes. In: Plant and Animal Genome XX Conference; 14-18 January 2012; San Diego. 2012.

39. Matvienko M, Kozik A, Froenicke L, Lavelle D, Martineau B, Perroud B, Michelmore R: Consequences of normalizing transcriptomic and genomic libraries of plant genomes using a duplex-specific nuclease and tetramethylammonium chloride. PLoS ONE 2013, 8:e55913.

40. Zhulidov PA, Bogdanova EA, Shcheglov AS, Vagner LL, Khaspekov GL, Kozhemyako VB, Moroz LL, Lukyanov SA: Simple cDNA normalization using kamchatka crab duplex-specific nuclease. Nucleic Acids Res 2004, 32:e37–e37.

41. Goecks J, Nekrutenko A, Taylor J: Galaxy: a comprehensive approach for supporting accessible, reproducible, and transparent computational research in the life sciences. Genome Biol 2010, 11:R86.

42. R Development Core Team: R: A Language and Environment for Statistical Computing. Vienna: 2010. http://www.R-project.org.

43. Bayer M, Milne I, Stephen G, Shaw P, Cardle L, Wright F, Marshall D: Comparative visualization of genetic and physical maps with Strudel. Bioinformatics 2011, 27:1307–1308.

doi:10.1186/1471-2164-15-945
Cite this article as: Hulse-Kemp et al.: Development and bin mapping of gene-associated interspecific SNPs for cotton (Gossypium hirsutum L.) introgression breeding efforts. BMC Genomics 2014 15:945.

Submit your next manuscript to BioMed Central and take full advantage of:

• Convenient online submission
• Thorough peer review
• No space constraints or color figure charges
• Immediate publication on acceptance
• Inclusion in PubMed, CAS, Scopus and Google Scholar
• Research which is freely available for redistribution

Submit your manuscript at www.biomedcentral.com/submit