Research Article

Genome Microscale Heterogeneity among Wild Potatoes Revealed by Diversity Arrays Technology Marker Sequences

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Tuber-bearing potato species possess several genes that can be exploited to improve the genetic background of the cultivated potato Solanum tuberosum. Among them, S. bulbocastanum and S. commersonii are well known for their strong resistance to environmental stresses. However, scant information is available for these species in terms of genome organization, gene function, and regulatory networks. Consequently, genomic tools to assist breeding are meager, and efficient exploitation of these species has been limited so far. In this paper, we employed the reference genome sequences from cultivated potato and tomato and a collection of sequences of 1,423 potato Diversity Arrays Technology (DArT) markers that show polymorphic representation across the genomes of S. bulbocastanum and/or S. commersonii genotypes. Our results highlighted microscale genome sequence heterogeneity that may play a significant role in functional and structural divergence between related species. Our analytical approach provides knowledge of genome structural and sequence variability that could not be detected by transcriptome and proteome approaches.

1. Background

The subgenus Potatoe of the Solanaceae family includes approximately 188 tuber-bearing species [1]. They display large ecological adaptation encompassing several traits that are lacking in the commercial potato and useful for breeding [2]. Among wild potato species, Solanum bulbocastanum Dun. and S. commersonii Dun. ex Poir. have attracted the attention of researchers and breeders. S. bulbocastanum is a known source of resistance to late blight disease of potato, and four late blight resistance genes have been cloned from this species to date [3–7]. S. commersonii ranks first among Solanums in terms of cold tolerance and capacity to cold acclimate, and it is also a source of resistance to pathogens such as Ralstonia solanacearum and Pectobacterium carotovorum [8, 9]. S. bulbocastanum and S. commersonii are among approximately 20 diploid potato species classified as superseries Stellata by Hawkes [10]. Despite their importance as sources of genes for crop improvement, relatively few genetic and genomic resources are available for these species, and little is known on their genome organization, gene function, and regulatory networks. Recently, a Diversity Arrays Technology (DArT) array was constructed for potato [11]. The array contains markers derived from various Solanum species, including S. bulbocastanum and S. commersonii. DArT arrays offer the potential to simultaneously survey large numbers of anonymous loci distributed throughout the genome. DArT markers are highly transferrable across populations or even across species, since the DArT array comprises a structured marker set that is surveyed in each experiment. Importantly, polymorphic DArT markers correspond to a set of DNA clones that can be sequenced for downstream applications.

The availability of the potato DArT array together with the recent release of the complete genome sequences of cultivated potato [12] and tomato [13] provide an attractive opportunity for comparative genomic studies aimed at understanding genome evolution at the species level. The genomes of potato and tomato are largely syntenic, and molecular
markers and gene content are predominantly conserved [13–16]. This degree of similarity has already enabled cross species comparative genomic approaches for gene mapping and cloning, reviewed by Bradeen [17]. Bioinformatics platforms improve community access to these resources and related omics collections, playing an important role for data mining and genome integration [18, 19]. In contrast to this wealth of knowledge and resources for cultivated potato and tomato, very little is known about genome structure and gene content in the wild relatives of potato.

In this paper, we exploited the reference genome sequences of potato and tomato and a collection of sequences of potato DArT array markers that show polymorphic representation across the genomes of S. bulbocastanum and/or S. commersonii genotypes. Our aim was to define a preliminary collection of marker sequences informative for the two species as a starting point for investigation of genome structure. This collection was also useful to highlight microscale genome sequence heterogeneity that possibly plays a meaningful role in functional and structural divergence between related species.

2. Materials and Methods

2.1. Plant Materials and DArT Marker Analyses. Two genotypes of Solanum bulbocastanum and two genotypes of Solanum commersonii were analyzed in this study. S. bulbocastanum genotypes include PT29 (P1243510), a source of the late blight resistance gene RB [3], and G15 (P1255516), a source of the RB locus allele RB-rc [20]. The S. commersonii genotypes include the frost tolerant cmm1T (P1243503) [8] and cmm6-3 (P1590886), a seedling genotype selected based on its crossability with cmm1T [21]. Total genomic DNA of genotypes is referred to as BLB- and CMM-specific markers, respectively. The genome sequence of S. bulbocastanum and/or S. commersonii genotypes and 550 hybridized in a polymorphic fashion with S. bulbocastanum genotypes and 550 hybridized in a polymorphic fashion with S. bulbocastanum genotypes. Hereafter, these markers will be referred to as BLB- and CMM-specific markers, respectively.

DNA sequencing of inserts was completed at the University of Minnesota BioMedical Genomics Center using BigDye Terminator (Applied Biosystems) cycle sequencing on an Applied Biosystems 3100 or 3700 automatic sequencer. Each sequencing reaction contained 1 μL of purified PCR product and 3.2 pmol of DArT-M13f or DArT-M13r. Each insert was sequenced in both directions in separate reactions. Resulting sequences were trimmed of vector and assembled into consensus sequences using SeqMan, part of the DNASTAR (Madison, WI) Lasergene software package.

Out of 1,423 DArT marker clones sequenced, 756 hybridized in a polymorphic fashion with S. bulbocastanum genotypes and 550 hybridized in a polymorphic fashion with S. commersonii genotypes. Hereafter, these markers will be referred to as BLB- and CMM-specific markers, respectively. The remaining 117 DArT markers hybridized and were polymorphic in both species (indicated as BLB/CMM).

2.2. Sequence Analysis and Data Interpretation. The genome sequence of Solanum phureja [12] served as the reference genome for our analyses. The genome sequence of Solanum lycopersicum [13], another reference species among Solanaceae, was also employed. For both genomes, our analyses
The majority of the 1,423 DArT 3.1 Dataset Description. Result and Discussion

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markers relative to the tomato genome. The discrepancy and 78% of BLB, 81% of CMM, and 76% of BLB/CMM DArT markers relative to the potato genome

length (data not shown).

About 92% and 79% of all DArT sequences could be aligned the potato and tomato genomes, respectively (Table 1). These comprise 93% of BLB, 91% of CMM, and 90% of BLB/CMM DArT markers relative to the potato genome and 78% of BLB, 81% of CMM, and 76% of BLB/CMM DArT markers relative to the tomato genome. The discrepancy

between the percentage of alignments to each genome is consistent with the composition of the reference potato DArT array that emphasizes markers from Solanum species more closely related to potato [11].

Sequence alignments were grouped into six categories, as described in Section 2. In the alignments to both potato and tomato genomes, DArT markers most frequently occurred as group (1) solitary one match, with 344 and 321 matches for potato and tomato, respectively, and as group (4) overlapping in uniform groups—one match, with 755 matches for potato and 663 matches for tomato (Figure 1(a)). For alignments to the potato genome, these two categories encompass 84% of all sequenced DArT markers: 82% for BLB, 87% for CMM, and 88% for BLB/CMM (Figure 1(b)). For alignments to the tomato genome, these same categories comprise 88% of all DArT marker sequences: 84% for BLB, 91% for CMM, and 91% for BLB/CMM. The remaining four marker alignment categories each represent less than 10% of the total number of aligned DArT marker sequences (Figure 1(b)). Briefly, groups (2) solitary multiple-matches and (5) overlapping in uniform groups—multiple matches show alignment to more than one genome region; this is probably due to repeated regions in the genome sequence; therefore, we considered these markers to be redundant. Groups (3) mixed and (6) overlapping in heterogeneous groups comprise DArT sequences with different alignment configurations probably due to intrinsic sequence properties. DArT marker sequences assigned to categories (1) and (4) overlap in unique regions in both the potato and tomato genomes. Since these markers are associated unambiguously to specific genome locations, they were considered as nonredundant markers and were subjected to further analyses; DArT markers not assigned to alignment categories (1) and (4) were not considered further.

3. Results and Discussion

3.1. Dataset Description. The majority of the 1,423 DArT sequences analyzed have a length ranging between 350 and 850 nucleotides, providing a consistent dataset for subsequent bioinformatics analyses. In particular, 68% of BLB markers and 73% of CMM markers are 450 to 700 nucleotides in length (data not shown).

Table 1: Results of DArT alignments to potato and tomato reference genomes. For each collection, the total number of DArT markers and the number (%) of aligned DArT markers are reported.

| Collection | Total no. of DArT | No. aligned (%) to Potato | No. aligned (%) to Tomato |
|------------|-------------------|---------------------------|--------------------------|
| BLB        | 756               | 703 (92.9)                | 586 (77.5)               |
| CMM        | 550               | 499 (90.7)                | 446 (81.1)               |
| BLB/CMM    | 117               | 105 (89.7)                | 89 (76.1)                |
| All        | 1423              | 1307 (91.8)               | 1121 (79.0)              |

Fifty-three DArT marker sequences that did not align to either the potato or tomato genome sequences based on the GenomeThreader approach were assembled using CAP3 [26] (parameters: -p 40 -o 80) before a second alignment attempt based on BLASTn [27] (parameters: -e 0.003). These same DArT sequences were also aligned to the GenBank nucleotide collection (nr/nt) using BLASTn and to the nonredundant protein sequences dataset using BLASTp [28]. A BLAST2GO analysis [29, 30] was performed to classify genes associated to DArT marker sequences to show the cellular, biological, and molecular functional information of the subset annotation.

3.2. Analysis of Nonredundant DArT Markers. In total 1,099 and 984 nonredundant (i.e., group (1) and group (4)) DArT marker sequences align to the potato and tomato genome sequences, respectively. The majority of the marker sequences align with a sequence identity exceeding 80% and a coverage greater than 90% (Figure 2). The percentage of alignments in the highest coverage category (between 90 and 100%) is 92% for potato and 75% for tomato. Many of the alignments overlap gene regions in both genomes (Figure 2). This is not unexpected since DArT markers are obtained through digestion by PstI. PstI is a methylation-sensitive enzyme; therefore, it is possible that it acts mainly on hypomethylated
Total 1423

Aligned 1307–1123

Not aligned 116–302

Overlapping in uniform groups 822–701

Solitary 420–364

Solitary one match 344–321

Solitary multiple matches 76–43

Mixed 32–17

Overlapping in uniform groups—
one match 755–663

Overlapping in uniform groups—
multiple matches 67–38

Overlapping in heterogeneous groups 33–40

(1) Potato

(2) Tomato

(3) All

(4) BLB

(5) CMM

(6) BLB/CMM

Figure 1: Categories of DArT markers alignments. (a) Values represent the number of alignments along the potato and tomato genome, respectively. (b) Pie charts of the percentage of aligned DArT markers, for each collection. The colour code is associated to the coloured rectangles of (a) and percentages are reported only when greater than 10%.

Figure 2: DArT marker sequences align predominantly with gene coding regions of the potato and tomato genome. The alignments associated (or not) to a gene locus along the potato and tomato genomes are highlighted in red (or blue). For each group, the number of alignments is also given.
DNA which, in turn, may correspond to gene regions, which are typically hypomethylated [31]. In Figure 3, the BLAST2GO analyses of the genes overlapping DArT marker regions are shown for both potato and tomato annotations. In particular, the figure shows the overrepresentation of genes associated with catalytic and binding activities.

In percentage, the two marker groups (1 and 4) represent 84% and 88% of all marker sequences aligned to the potato and the tomato genomes, respectively. Interestingly, in contrast with average results across all DArT sequences (Table 1) showing more matching DArT sequences to potato than to tomato, a higher proportion of the nonredundant groups align to the tomato genome than to the potato genome. This may be due to the higher contribution of ambiguous alignments (group (2) and (5)) in potato. This in turn suggests a higher sequence repetitiveness in the potato genome or better sequence quality for the tomato genome [12, 13]. Overall, nonredundant DArT marker sequences show very high coverage in potato compared to tomato (Figure 2), confirming higher phylogenetic similarity amongst potato species.

We next examined total coverage of the genome sequences from cultivated potato and tomato represented by alignments with DArT marker sequences (Table 2). Details per chromosomes are reported in the supplementary Table S1 (see Table S1 in Supplementary Material available at http://dx.doi.org/10.1155/2013/257218). In general, BLB DArT markers encompass a greater number of nucleotides in each genome than CMM or BLB/CMM markers. This is not surprising since BLB markers are the largest subset of DArT markers examined in this study. BLB DArT markers represent 208.8 Kbp of the potato genome but only 175.8 Kbp of the tomato genome. In contrast, CMM and BLB/CMM markers represent approximately equivalent regions of the potato and tomato genomes (CMM: 137.9 Kbp for potato versus 139.6 Kbp for tomato; BLB/CMM: 29.4 Kbp for potato versus 24.7 Kbp for tomato). We further divided the nonredundant DArT markers into two subclasses. Common markers align with the genome sequences of both potato and tomato; specific markers align to only one of the two genomes (Table 2). Within each sub-class, alignments were either ungapped (i.e., marker sequences aligned to genome sequences without disruption) or gapped (i.e., marker sequences aligned to genome sequences but alignments were interrupted by genome sequence not found in marker sequences). It is noteworthy that the same DArT marker sequence could be ungapped when aligned to the potato genome and gapped when aligned to the tomato genome or vice versa. The relative ratio of gapped versus ungapped regions of all BLB, CMM, and CMM-BLB DArT marker sequences relative to the potato and tomato genome sequences provides insight into patterns of genome evolution and species relationships. Distinction between gapped and ungapped alignments is necessary since variability in the length of gapped markers can complicate interpretation of the degree of genome coverage by the marker sequences. In potato, for example, the size of most of the gaps (89%) ranges from 20 to ~1000 bps. The remaining ones reach a maximum at ~5000 bps (not shown). For common DArT markers, the contribution of ungapped regions to total genome representation is higher in potato than in tomato for each marker collection. In contrast, for common markers, the contribution of gapped regions is generally lower in potato than in tomato. This again reflects higher phylogenetic similarity of the wild species to the cultivated potato. However, it is interesting to note that the relative frequency of common gapped regions compared to common ungapped ones in potato versus tomato is comparable for both BLB (14.21% in potato and 16.68% in tomato) and BLB/CMM (5.14% potato and 3.16% in tomato) DArT markers. The frequency of CMM common gapped and ungapped regions differs in potato (7.73%) with respect to tomato (15.64%). This indicates that, in contrast to BLB markers, CMM markers align with fewer gaps to the potato genome sequence than to the tomato genome sequence. This implies that the genomes of S. commersonii and potato are more similar at a DNA sequence level than are the genomes of S. bulbocastanum and potato, consistent with S. commersonii being phylogenetically more closely related to potato than is S. bulbocastanum, as the analyses based on plastid genomes previously suggested [32–34].

Considering the contribution of specific DArT markers, ungapped BLB markers provided the greatest overall genome coverage for both potato and tomato, consistent with higher representation of BLB markers in our dataset (Table 2). Importantly, the relative proportion of gapped regions compared to ungapped regions for the specific alignments indicates a comparable behaviour in the three marker collections in both species.

3.3. Genome Sequence Heterogeneity. We compared marker origins and alignment classifications across the potato and tomato genomes (Table 3). In general, the majority of aligned DArT markers are ungapped in both potato and tomato: 328 (77%) for BLB, 297 (83%) for CMM, and 65 (91%) for BLB/CMM. Eight BLB and 16 CMM markers align to both genomes in a gapped configuration (Table 3). Interestingly, a high percentage of aligned markers exhibit heterogeneous behaviours across the potato and tomato genomes (i.e., gapped versus ungapped in potato versus tomato and vice versa). These sequences are a source of marker variability between wild and cultivated species that can be exploited in future studies.

Seven BLB, 16 CMM, and one BLB/CMM markers aligned to the genomes of both potato and tomato in a gapped configuration (Table 3). As shown in Table S2, each of the seven BLB DArT markers aligned to gene regions in both species. Among these, five regions corresponded to genes with identical annotations in potato and tomato. On the other hand, among the 16 CMM DArT markers, only 10 and 14 aligned to gene coding regions in potato and tomato, respectively. Of the 10 CMM markers aligning to both potato and tomato gene coding regions, all of the 10 aligned to regions with identical gene annotations in both species (Table S2).

Nine DArT markers, three from BLB and six from CMM, aligned with the same alignment structure (i.e., number and length of gapped and ungapped regions) to homologous chromosomes in both potato and tomato and to gene loci with the same annotation (Table S2). The remaining two BLB, four
Figure 3: BLAST2GO analyses of the genes overlapping DArT marker regions.
Table 2: Number of nucleotides (in Kbp units) covered by DArT alignments. For details on coverage categories, see Section 2.

| Coverage category | Potato | Tomato |
|-------------------|--------|--------|
|                   | BLB    | CMM    | BLB/CMM | BLB    | CMM    | BLB/CMM |
| Common            |        |        |         |        |        |         |
| Ungapped          | 132.3  | 97.7   | 20.5    | 128.2  | 95.3   | 19.1    |
| Gapped            | 18.8   | 7.6    | 1.1     | 21.4   | 14.9   | 0.6     |
| Specific          |        |        |         |        |        |         |
| Ungapped          | 46.1   | 22.3   | 7.7     | 22.5   | 17.3   | 4.4     |
| Gapped            | 10.2   | 10.4   | 0.2     | 3.7    | 12.1   | 0.7     |
| Total             | 208.8  | 137.9  | 29.4    | 175.8  | 139.6  | 24.7    |

Table 3: Comparison between DArT alignments to potato and tomato genomes. Number of DArT markers aligned along the potato (horizontal) and tomato (vertical) genomes for each collection, given in parenthesis. Each cell, within each matrix, shows the number of DArT markers per alignment type: ungapped, gapped, or not aligned.

CMM, and one BLB/CMM markers, although aligning to homologous chromosomes in genes of the same annotation, showed heterogeneous (i.e., number and length of gapped and ungapped regions) alignment structure (Table S2). These observations of microscale genome heterogeneity may be relevant to investigation of genome structures, functionalities, and properties of the represented Solanum species.

3.4. DArT Marker Sequences Not Aligned to the Reference Genomes. Some DArT markers could not be aligned to one or both genome sequences (Table 1). In particular, 116 marker sequences could not be aligned to the potato genome, 302 marker sequences could not be aligned to the tomato genome, and 51 marker sequences could be aligned to neither to potato nor to tomato. These were selected as putative wild species-specific markers and were assembled using the CAP3 software, yielding seven assembled consensus sequences comprising 20 sequences in total. The remaining 31 DArT marker sequences could not be assembled. Next, we attempted a less stringent alignment of the resulting 38 sequences (31 unassembled sequences plus seven consensus sequences) to the potato and the tomato genome sequences using the BLASTn algorithm (Table S3). Using this approach, 18 DArT marker sequences could be assigned to single locations in both the potato and tomato genomes, and only nine markers aligned to multiple genome locations in one or both species. In these cases, the less stringent alignment search performed by the BLAST software helped to confirm the presence in the potato and tomato genomes of 27 DArT marker sequences, previously unidentified in the more stringent GenomeThresher analysis. Moreover, in some cases, the BLASTn analysis confirmed matches to the same chromosome for both potato and tomato (e.g., DArT markers 472847 (chromosome 1), 537586 (chromosome 8), 473780 (chromosome 2), and 534573 (chromosome 11)). The presence of low level sequence similarity between these markers and the potato or tomato genome sequences revealed distant relationships between the wild and cultivated species and may be exploited in the study of cross-species genome heterogeneity. Twenty-two DArT markers (Table S3) showed extreme repetitive distribution along the potato and tomato chromosomes and were described by ambiguous annotations. Nevertheless, protein-based annotations (BLASTp), when present, generally confirmed homology with Solanum proteins or with those from more distantly related plant species. Two DArT marker sequences failed to align to the genomes of either potato or tomato even under more permissive analytical criteria.

4. Conclusions

Potato (S. tuberosum) and tomato (S. lycopersicum) belong to the subgenus Potatoe of the large and diverse genus Solanum. Although horticulturally distinct, potato and tomato share a clear evolutionary history that is well supported by molecular
data [35, 36]. The species are thought to have diverged from a common ancestor approximately 6.2 to 7.3 million years ago [37, 38]. Sexual isolation and subsequent divergence of the two species were accompanied by a series of structural genomic changes including chromosome arm inversions and large-scale translocations [14, 15]. Nevertheless, the genomes of potato and tomato are largely syntenic and molecular marker and gene content are predominantly conserved [14–16]. This degree of similarity has enabled cross species comparative genomics approaches for gene mapping and cloning, reviewed by Bradeen [17], efforts that will likely be furthred by the recent release of the complete genome sequences of potato [12] and tomato [13].

In this study, we proposed a suitable methodology to exploit partial genome information from wild species in the presence of reference genomes from related species. This approach, here exploited with DArT marker sequences, can also be employed in partial genome resequencing or similar efforts. Our results also highlighted the presence of divergent sequence relationships and heterogeneous alignment structures, including the presence/absence of gaps, which are detectable thanks to appropriate, less stringent comparative methods. This divergence commonly occurred even in gene pairs with apparent orthologous relationships and presumed functional conservation, and it could often be confirmed both in potato and tomato genomes. Evidence from results supported by two reference-related species partially overcomes possible limits that may be due to the quality of first released genomes and suggests a fine microscale genome structural divergence between wild and cultivated species in the Solanaceae. Our results confirm the utility of suitable analysis of the tuber crop potato,” Nature, vol. 475, no. 7355, pp. 189–195, 2011.

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Conflict of Interests
The authors declare no conflict of interests.

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