Comparative Analysis of Gene Expression by Microarray Analysis of Male and Female Flowers of Asparagus officinalis

Wu-Jun GAO,1 Shu-Fen LI,1 Guo-Jun ZHANG,2 Ning-Na WANG,1 Chuan-Liang DENG,1 and Long-Dou LU1,

1College of Life Sciences, Henan Normal University, Xinxing 453007, China
2Department of Immunology, Xinxing Medical University, Xinxing 453003, China

Received December 7, 2012; Accepted March 4, 2013; Online Publication, June 7, 2013

To identify rapidly a number of genes probably involved in sex determination and differentiation of the dioecious plant Asparagus officinalis, gene expression profiles in early flower development for male and female plants were investigated by microarray assay with 8,665 probes. In total, 638 male-biased and 543 female-biased genes were identified. These genes with biased-expression for male and female were involved in a variety of processes associated with molecular functions, cellular components, and biological processes, suggesting that a complex mechanism underlies the sex development of asparagus. Among the differentially expressed genes involved in the reproductive process, a number of genes associated with floral development were identified. Reverse transcription-PCR was performed for validation, and the results were largely consistent with those obtained by microarray analysis. The findings of this study might contribute to understanding of the molecular mechanisms of sex determination and differentiation in dioecious asparagus and provide a foundation for further studies of this plant.

Key words: Asparagus officinalis; differentially expressed genes; dioecious plant; microarray; sex determination and differentiation

Most flowering plants are hermaphroditic, with bisexual flowers that contain both male and female reproductive organs. Only approximately 6% of angiosperm species are dioecious plants, with unisexual flowers formed in distinct individuals, staminate flowers in male plants and pistillate flowers in female ones.1) There are many different sex-determination mechanisms in plants, ranging from the XY system common to many species to a completely autosomal determination system.2–6) Knowledge of sex determination in plants remains limited, although much progress has been made in the study of other aspects of dioecious plants, such as sex-linked genes over the past years.7–13)

Among dioecious plants, garden asparagus (Asparagus officinalis) belongs to the family Liliaceae, with 2n = 2x = 20 chromosomes and a haploid genome size of 1.323 Mb.14) The chromosomes have been classified as 5L (long), 1M (medium), and 4S (small) based on chromosome size and Giemsa C banding pattern.15) The L3 chromosomes have been identified as sex chromosomes by analysis of segregation patterns in a series of trisomic lines.16) Female asparagus plants are homogametic (XX), while the male plants are heterogametic (XY), but the X and Y chromosomes do not differ in morphology (e.g., homomorphism). It is nearly impossible to distinguish male from female plants morphologically during the vegetative growth stage, since morphological differences are observed only in the structures of the floral organs. In animals, the germ cell line is differentiated in early development, while plants have no distinct germ cell line.17) Like other dioecious plants, asparagus also has a hermaphroditic stage. At this stage, floral meristems in both males and females are potentially hermaphroditic in early flower development, followed by differential abortion or arrest of sex organs occurring at a variety of stages.18,19) During the development of unisexual flowers, the development and differentiation of the stamen and ovary are directly or indirectly controlled and regulated by a number of genes.20) The characterization and comparison of gene expression patterns between male and female asparagus plants is fundamental to understand the complex processes underlying sex determination and differentiation. Presently, only a few genes have been identified in asparagus,18,21–23) and there have been no studies involving a systematic comparison of gene expression between male and female asparagus plants. It is still unknown how many genes are responsible for sex determination and differentiation in flowers, and which pathway or factor regulates sex determination and differentiation in this dioecious plant species. To address these questions, it is necessary to identify genes whose expression patterns are sex-biased in asparagus reproductive organs.

Among the various techniques used for gene expression profiling, fluorescence-based microarray analysis, which can simultaneously measure the expression of thousands of genes and identify subtle changes in gene expression levels, is widely accepted as a rapid and efficient tool for the comprehensive characterization of global gene expression profiles at diverse stages of plant development.24) Hence, the present study used micro-

1 To whom correspondence should be addressed. Tel: +86-0373-3326341; Fax: +86-0373-3329102; E-mail: lld5910@yahoo.com
Abbreviations: EST, expressed sequence tag; FDR, false discovery rate; GO, gene ontology; PCA, principal component analysis; RIN, RNA integrity number; RT-PCR, reverse transcription-PCR
array analysis for a comparative analysis of differentially expressed genes in the male and female flowers of asparagus. Several differentially expressed genes were further validated by RT-PCR. The findings on sex-biased expression genes in this study should provide a platform for further study of the sex determination and differentiation mechanisms of asparagus.

Materials and Methods

Plant materials. Asparagus officinalis variety UC509 was used. The plants were grown in a greenhouse at Henan Normal University. The whole flower organ was sampled separately from the male and female plant derived from the full-sibling progeny. Thirty flowers buds (early developmental stage, about 0.7 mm of the whole flower length) 25) per individual plant were taken as samples for RNA extraction and subsequent microarray analysis.

RNA extraction. Total RNA was isolated from samples prepared from male and female asparagus plants using TRIZOL reagent (Life Technologies, Carlsbad, CA) following the manufacturer’s instructions. The RNA integrity number (RIN) was verified by Agilent Bioanalyzer 2100 (Agilent Technologies, Santa Clara, CA). The total RNA samples were further purified using an RNeasy mini kit (Qiagen, Hilden, Germany).

Agilent gene-chip construction and hybridization. Agilent microarray construction and hybridization were carried out by Shanghai Biotechnology (Shanghai, China). Asparagus gene chips were designed based on sequences deposited in the asparagus EST database at NCBI (Agilent design ID, 036397). Each customized chip contained spots in triplicate with 8,665 EST-specific 60-mer oligonucleotides. The purified RNA sample was amplified and labeled with a Cy3 dye by following the manufacturer’s instructions. Then the slides were washed in staining dishes (Thermo Shandon, Waltham, MA) using a wash buffer kit (Agilent) following the manufacturer’s instructions. Then the slides were scanned on an Agilent Microarray Scanner (Agilent) with default settings (green dye channel, scan resolution 5 μm, PMT 100% and 10%, and 16 bit scans). Finally, raw expression data obtained using Feature Extraction software 10.7 (Agilent) were normalized by quantile algorithm with Gene Spring Software 11.0 (Agilent).

Bioninformatics analyses. Fold change, Diffgene, t-test, principal component analysis (PCA), heat map, and GO enrichment analyses were performed by means of the SBC Analysis System (http://sas.ebioservice.com) designed by Shanghai Biotechnology.

Reverse transcription-PCR. Reverse transcription-PCR (RT-PCR) was used to validate the microarray data. A set of differentially expressed genes identified by microarray analysis were selected for this validation. Total RNA samples were prepared from flowers and leaves of male and female asparagus individuals for this test so as to compare expression patterns between the reproductive and vegetative organs.

The RNA samples were treated with RNase-free DNase I (Takara, Dalian, China) for 30 min at 37 °C and subsequently purified. 3 μg of RNA from each sample was used for first-strand cDNA synthesis with a reverse transcription kit (Toyobo, Osaka, Japan). The specific primers for the eight target genes from asparagus are listed in Table 1. Housekeeping gene actin was selected as a reference gene in the RT-PCR reactions using primer pair cx909 and cx910, described previously.26) The reaction program included denaturation at 95 °C for 5 min, 30 cycles of 95 °C for 30 s, 60 °C for 30 s, 72 °C for 1 min, and final incubation at 72 °C for 7 min. The intensity of the bands of the amplimers was measured after electrophoresis, which were then stained to assess the expression levels of the target genes.

Results

Transcriptome profiling analysis of male and female asparagus flowers

To examine the differentially expressed genes in the flowers of male and female asparagus plants, an Agilent customized asparagus microarray containing 8,665 probes was designed and employed in gene expression profiling. Principal component analysis (PCA) was performed to characterize the relationships of the six samples to obtain an overview of gene expression profiles and to evaluate the quality of the data. As shown in Fig. 1, samples were plotted in correlation with the first two principal components responsible for 96.99% of the total variation in gene expression. Data spots from the same gender were clustered together, suggesting that the data quality obtained by the gene chip was high enough to reveal gene expression differences between male and female flowers in the asparagus plants.

SBC Diffgene analysis was performed using the microarray data for male and female flowers to identify differentially expressed genes. Among the 8,665 ESTs

<table>
<thead>
<tr>
<th>Gene name</th>
<th>Primer (5'-3')</th>
<th>GenBank number</th>
<th>Male-/Female-biased</th>
<th>Fold change</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ao1</td>
<td>L: GGAGAGCTGAGTACATTGC</td>
<td>CV291163</td>
<td>Male</td>
<td>38.815</td>
</tr>
<tr>
<td></td>
<td>R: GCAGCAAGTGGAGAGGTGGA</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ao2</td>
<td>L: GCAGGACATACAGCCAG</td>
<td>CV287736</td>
<td>Male</td>
<td>30.823</td>
</tr>
<tr>
<td></td>
<td>R: CGTCCGCTTCTCATGGT</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ao3</td>
<td>L: GTGGTGTCATGCGGCACTTC</td>
<td>CV291009</td>
<td>Male</td>
<td>9.251</td>
</tr>
<tr>
<td></td>
<td>R: GAGATGCTAAACATCCGGAA</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ao4</td>
<td>L: GCTGGTTGCGTCTGGAAACGATC</td>
<td>CV288431</td>
<td>Male</td>
<td>10.929</td>
</tr>
<tr>
<td></td>
<td>R: GATGGGCCATGTTGAAAGAC</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ao5</td>
<td>L: TCTGTTGCTGGTGGCATG</td>
<td>CV288445</td>
<td>Female</td>
<td>7.088</td>
</tr>
<tr>
<td></td>
<td>R: ATGGCGTAAACTTTGTCGC</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ao6</td>
<td>L: AGACCAAACACCAATAG</td>
<td>CV291750</td>
<td>Male</td>
<td>1259.957</td>
</tr>
<tr>
<td></td>
<td>R: CTAATACCTCTCTACTACCTCC</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ao7</td>
<td>L: CGGACATAATACGTCGCCTTG</td>
<td>CV290351</td>
<td>Male</td>
<td>213.239</td>
</tr>
<tr>
<td></td>
<td>R: CAATGCTAGAGGCTCTTTCG</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ao8</td>
<td>L: CATTGGCTGCTGGTCTGTA</td>
<td>CV458582</td>
<td>Female</td>
<td>7.130</td>
</tr>
<tr>
<td></td>
<td>R: CTTTTGCTCATCTCTAGTCG</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Actin</td>
<td>L: CAAATCGTGAGAGAGATACCCA</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>R: CCATGAGGAAGCTCAGTACTCT</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 1. Primers Used and Basic Information on Selected Genes by RT-PCR Analysis
tested, 1,948 ESTs were found to be differentially expressed (fold change >2, p value <0.05 and q value <0.05). These differentially expressed ESTs represent 1,181 genes expressed in male and female asparagus flowers. Of them, 980 ESTs were male-biased, representing 638 unigenes, and 968 ESTs were female-biased, representing 543 unigenes. A total of 66 male-biased ESTs showed expression changes by more than 10-fold, with the largest change by 47.4-fold, whereas only 10 female-biased ESTs exhibited more than 10-fold changes in expression, with the largest change by 1,259.96-fold.

Figure 2 shows the results of heat-map and clustering analysis of the comparison between the 638 male-biased and 543 female-biased genes. In accord with the PCA analysis, the overall gene expression profiles for the three male flower samples were similar, and gene expression in the three female flower samples was similar too.

Furthermore, we examined the microarray data hoping to identify putative male and female-specific genes, since such genes might play important roles in the sex determination and differentiation of dioecious plants. Unfortunately, no male or female-specific genes were identified by this analysis, although a number of genes showed highly divergent expression levels as between male and female flowers.

Gene ontology analysis of differentially expressed genes
To investigate further the biological processes involved in the sex determination and differentiation of asparagus, a gene ontology (GO) enrichment analysis was performed to determine whether the differentially expressed genes were significantly associated with GO terms and functional subsets related to specific biological processes. Male- and female-biased genes with significance at p < 0.05 in the GO enrichment test were considered for further analysis. The male- and female-biased genes and their associated GO terms are shown in Fig. 3. They were found to be related to various processes involving molecular functions, cellular components, and biological processes. This indicates that the sex determination and differentiation of asparagus is very complex, and that numerous genes and molecules are involved in this process.

A number of differentially expressed genes were clustered into molecular function categories transcription regulator activity and translation regulator activity, and the biological process categories developmental process and reproduction. These genes may be important in sex determination and differentiation in asparagus plants. Another set of differentially expressed genes was clustered in general categories, such as binding, cell, organelle, cellular process, and metabolic process. The genes included in these categories did not show any obvious association with specific molecular pathways. A group of differentially expressed genes was classed into
the categories response to stimulus and immune system process, suggesting that some genes responsive to different stimuli, such as biotic and abiotic stresses, can also function in the divergence between male and female asparagus plants. Furthermore, the genes clustered into categories nutrient reservoir activity and death included only male-biased expression genes. Notably, male-biased genes were associated with more GO terms than female-biased genes, and almost every GO term included more hits on male-biased genes. This suggests that the development of the male flower involves more processes and molecules than female flowers in this plant species.

More importantly, 36 male- and 39 female-biased expression genes were found to be involved in the reproductive process. Clearly, these differentially expressed reproduction-related genes are very important to the development of male and female flowers and the sex determination or differentiation of asparagus plants. Among these reproduction-related genes, several unigenes were found to be putative homologs of Arabidopsis genes involved in floral development (Table 2). The proteins encoded by these genes included transcription factors such as SEPALLATA2 (SEP2), SEPALLATA3 (SEP3), zinc finger family proteins, MADS-box genes, and flowering time control protein FPA. All these proteins were found to control floral development. However, these unigenes were not male or female-specific genes.

Validation of target gene expression profiles by RT-PCR analysis

To evaluate the authenticity of the microarray data, eight differentially expressed genes were selected for further analysis based on their expression levels and predicted functions. Specific primers were designed following the sequences of the genes, and their transcript levels were measured by RT-PCR. These genes were referred as Ao1-Ao8, respectively. Among them, Ao3, Ao4, Ao5, and Ao8 were selected because their predicted

---

**Fig. 3. Functional Classification of Differentially Expressed ESTs in Male and Female Asparagus Flowers.**

The distribution of differentially expressed ESTs between male and female asparagus flowers based on three levels, molecular function, cellular components, and biological process, as annotated through SBC GO analysis in relation to the gene ontology categorization of known Arabidopsis genes. The X axis represents numbers of ESTs, and the Y axis represents different GO terms.

<table>
<thead>
<tr>
<th>Molecular function</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Nutrient reservoir activity</td>
<td>Male-biased genes</td>
</tr>
<tr>
<td>Translation regulator activity</td>
<td></td>
</tr>
<tr>
<td>Transcription regulator activity</td>
<td></td>
</tr>
<tr>
<td>Enzyme regulator activity</td>
<td></td>
</tr>
<tr>
<td>Antiinflammatory activity</td>
<td></td>
</tr>
<tr>
<td>Electron carrier activity</td>
<td></td>
</tr>
<tr>
<td>Binding</td>
<td></td>
</tr>
<tr>
<td>Transporter activity</td>
<td></td>
</tr>
<tr>
<td>Structural molecule activity</td>
<td></td>
</tr>
<tr>
<td>Catalytic activity</td>
<td></td>
</tr>
</tbody>
</table>

| Cellular component                    |                   |
| Organelle                             |                   |
| Macromolecular complex                |                   |
| Extracellular                        |                   |
| Membrane-enclosed                   |                   |
| Cell                                 |                   |

| Biological process                    |                   |
| Negative regulation of biological process |                |
| Positive regulation of biological process |              |
| Biological regulation                 |                   |
| Multi-organism process                |                   |
| Localization                          |                   |
| Response to stimulus                  |                   |
| Rhythmic process                      |                   |
| Growth                                |                   |
| Developmental process                 |                   |
| Multicellular organellar process      |                   |
| Death                                |                   |
| Anatomical structure formation        |                   |
| Cellular process                      |                   |
| Metabolic process                     |                   |
| Immune system process                 |                   |
| Reproduction                          |                   |
functions were related to reproduction, and \textit{Ao1}, \textit{Ao2}, \textit{Ao6}, and \textit{Ao7} were selected for their large fold-change values. The primers and basic information for these selected genes are listed in Table 1. Among the eight genes selected, four were male-biased and four were female-biased. As shown in Fig. 4, the gene expression levels and patterns showed high similarity to the microarray data. Only one gene, \textit{Ao2}, exhibited similar expression levels in both male and female asparagus flowers. Furthermore, the expression levels of the eight genes selected were also analyzed in the leaves of male and female asparagus plants. All of the genes, except for \textit{Ao1} and \textit{Ao3}, showed equivalent expression levels in the leaves of male and female asparagus plants.

**Discussion**

Unlike the case of animals, no confirmed sex-determining gene has been identified in plants. However, a number of genes have been found to play roles in the development and differentiation of the stamen and ovary during the development of unisexual flowers.\(^2\)\(^1\)\(^1\) Gene regulation is also important in sex determination and differentiation in dioecious plants. Therefore, to understand sex determination and differentiation in dioecious plants, one approach is to isolate genes differentially expressed in male and female flower buds.\(^2\)\(^7\) This has led to the identification and characterization of several genes in the model dioecious species \textit{S. latiola}.\(^2\)\(^8\)\(^–\)\(^3\)\(^0\)

Transcriptome profiling was performed to identify differentially expressed genes as between male and female asparagus flowers via microarray analysis. Our work focuses on the early stage of flower development in view of the important roles of early flower genes in

\begin{table}[h]
\centering
\begin{tabular}{llccc}
\hline
Annotiation & Ath Gene ID & Male-/Female-biased & Fold change & FDR \\
\hline
Ubiquitin-conjugating enzyme E2 A (UBC2) & A2G02760 & Male & 3.085 & 0.0113 \\
Minor histocompatibility antigen H13 (SPP) & A2G03120 & Male & 5.970 & 0.0 \\
Developmental protein SEPALATA2 (SEP2) & A3G02310 & Male & 2.120 & 0.0229 \\
Exocyst complex component sec15B (SEC15B) & A4G02350 & Male & 2.651 & 0.0138 \\
Phosphoglucomutase 1 (PGI1) & A4G24620 & Male & 3.329 & 0.0022 \\
DDB1-and CUL4-associated factor-1 (DCAF1) & A4G31160 & Male & 2.410 & 0.0299 \\
Histone-lysine N-methyltransferase SETD1 (SDG25) & A5G14240 & Male & 6.579 & 0.0 \\
Protein WAX2 (CER3) & A5G57800 & Male & 11.192 & 0.0 \\
Histone-binding protein RBBP4 (MSI1) & A5G58230 & Male & 2.747 & 0.0089 \\
Developmental protein SEPALATA3 (SEPALLATA3) & A1G24260 & Male & 3.000 & 0.0089 \\
Endoribonuclease Dicer (DCL1) & A1G01040 & Male & 2.011 & 0.0343 \\
Auxin response factor (ARF6) & A1G30330 & Male & 3.067 & 0.0089 \\
GDSL-esterase/Lipase EXL3 (EXL3) & A1G75900 & Male & 3.086 & 0.0089 \\
Nuclear cap-binding protein subunit 1 (ABH1) & A2G13540 & Female & 2.133 & 0.0343 \\
Peptidyl-prolyl cis-trans isomerase CYP40 (SQN) & A2G15790 & Female & 2.822 & 0.0138 \\
Glycine-rich RNA-binding protein 7 (CCR2) & A2G21660 & Female & 2.078 & 0.0286 \\
Ethylene-responsive transcription factor RAP2-7 (RAP2.7) & A2G28550 & Female & 2.123 & 0.0343 \\
Squamosa promoter-binding-like protein 9 (SPL9) & A2G24220 & Female & 3.612 & 0.0138 \\
Flowering time control protein FPA (FPA) & A2G34140 & Female & 2.412 & 0.0229 \\
Agamous-like MADS-box protein AGL1 (SHP1) & A3G58780 & Female & 5.290 & 0.0022 \\
Actin-related protein 7 (ARP7) & A3G60830 & Female & 2.999 & 0.0089 \\
MADS-box transcription factor (AG) & A4G19890 & Female & 7.088 & 0.0022 \\
Putative rRNA-glutamine synthetase (OVA9) & A1G25350 & Female & 2.398 & 0.0229 \\
Auxin response factor 6 (ARF6) & A1G30330 & Female & 3.229 & 0.0139 \\
SEUSS transcriptional co-regulator (SEU) & A1G43850 & Female & 3.242 & 0.0113 \\
Protein BONSAL (BNS) & A1G73177 & Female & 3.203 & 0.0089 \\
Zinc finger protein-like protein (BTS) & A3G18290 & Female & 2.241 & 0.0343 \\
\hline
\end{tabular}
\caption{Comparison of Partial Genes Associated with Flower Development in Male and Female Asparagus Flowers. FDR, false discovery rate.}
\end{table}

\begin{figure}
\centering
\includegraphics[width=0.8\textwidth]{Fig4.png}
\caption{RT-PCR Analysis of Selected Differentially Expressed Genes in Male and Female Asparagus Flowers.}
\end{figure}

The gene names are listed on the right side of the picture. mf, male flower; ml, male leaf; ff, female flower; fl, female leaf; M, Molecular marker BM5000 (Biomed, Beijing, China).
mediating sex differentiation in dioecious plants. Transcriptome analysis via microarray data indicated that a complex molecular network is involved in early flower development in asparagus. This network includes a large set of transcriptional factors, signaling genes, and reproduction genes. A number of genes differentially expressed in male and female asparagus flowers were identified. Further functional analysis of these differentially expressed genes should provide insight into the molecular mechanisms underlying the sex determination and differentiation in asparagus plants.

The reproductive process, involving the activities of different types of transcription factors and genes, is one of the most important molecular events in dioecious plants. Our transcriptome profiling revealed a number of differentially expressed genes encoding transcriptional factors and proteins functioning in reproduction. Particularly, several differentially expressed genes associated with floral development were identified by comparison with Arabidopsis genes. These genes included SEP, MADS box genes, and zinc fingers family protein genes. For instance, MADS box genes participate in various steps of plant vegetative and reproductive development, including the most important phases of the reproductive process. The SEPALLATA class members SEP1, SEP2, SEP3, and SEP4 form a subfamily of MADS-box transcription factors that play critical roles in controlling the development of floral organs in flowering plants. In Arabidopsis, SEPALLATA class members specify the identities of all four whorls of floral organs. Similarly, expression of the FPA protein is required for flowering-time control, and is also implicated in RNA silencing. FPA was first identified through the characterization of a late-flowering Arabidopsis mutant. As a component of the autonomous pathway, FPA facilitates flowering by preventing accumulation of the mRNAs encoding transcription factor FLC. Further analysis of the putative homologs of these Arabidopsis genes in asparagus should reveal the functional conservation of these genes in asparagus, and might help to elucidate their roles in flower development in dioecious asparagus species.

Among differentially expressed genes detected here between male and female individuals, no male or female-specific genes were identified in the asparagus plants. To our knowledge, there is no report describing male or female-specific genes in asparagus, although several genes have been isolated from asparagus. One possible reason is that asparagus might have very small non-recombining sex-determining regions. Alternatively, these regions might have appeared at the early stages of sex chromosome evolution, resulting in the formation of few sex-specific genes. More investigation is needed to elucidate the molecular mechanisms involved in the sex determination and differentiation of asparagus plants.

RT-PCR analysis also indicated that most of the genes verified exhibited similar transcript levels in the leaves of male and female plants, but expression was different as between male and female flowers. As the leaves represent vegetative organs and the flowers represent reproductive organs, the expression patterns of the genes were similar during the vegetative stage but varied when entering the reproductive stage, suggesting that these genes play roles in flower development in asparagus. Although the selected genes identified by microarray analysis were confirmed by RT-PCR, further investigation is necessary to characterize each of these differentially expressed genes.

In this study, the microarray method was used to analyze differentially expressed genes in male and female flowers of asparagus. Although the total number of ESTs used in microarray analysis was limited, some useful information was obtained, indicating that even a limited EST dataset represents a valuable resource for genetic study of non-model organisms. Because the molecular mechanisms involved in the sex determination and differentiation of asparagus remain largely unknown, the large number of the differentially expressed genes identified in this study should serve as subjects in further studies to determine their functions and to dissect the molecular networks involved in sex determination and differentiation.

Acknowledgments

This work was supported by grants from the National Natural Science Foundation of China (grant nos. 30970211 and 31000165) and the Natural Science Foundation of Henan Province, China (grant no. 102300410043). We would like to thank Dr. Li Runzhi (Department of Plant and Soil Science, University of Kentucky) for review of this manuscript and constructive comments.

References

Microarray Analysis of Flowers of \textit{Asparagus officinalis} 1199