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Proteomic analysis of sugar beet apomictic monosomic addition line M14

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ABSTRACT

Apomixis in plants holds great promise for agriculture because of its vigor associated with heterozygosity and superior genotype. Despite the significance of apomictic reproductive process, our knowledge of proteins and their functions in apomictic development is limited. Here we report a comparative proteomic and transcriptomic analysis of sexual and apomictic processes in sugar beet. A total of 71 differentially expressed protein spots were successfully identified in the course of apomictic reproductive development using high-resolution 2-DE and MS analysis. The differentially expressed proteins were involved in several processes that might work cooperatively to lead to apomictic reproduction. This study has generated potential protein markers important for apomictic development.

1. Introduction

Apomixis is an asexual reproductive process by which plants produce seed without fertilization through female syngamy that produces embryos genetically identical to the maternal parent. It preserves heterozygosity vigor and maintains superior genotype through parthenogenetic embryo development. If the process of apomixis can be understood and harnessed, it will revolutionize genetic engineering and seed production for agriculturally important crops [1–3]. Therefore, apomixis has great potential for the utilization of heterosis in agriculture and for the study of plant reproductive biology. In spite of this widely recognized significance, very little is known about the genetic and developmental processes underlying the expression of apomixis [4,5]. Studying of the genetic control of traits in apomicts is usually complicated by the irregular segregation, polyploidy and lack of recombination. What is more challenging is the analysis of apomictic seed formation itself. This is because the processes taking place in the ovule and the gametophytic generation are represented by only a few cells during a very short period of time in the life cycle [6]. Although the developmental mechanisms underlying apomixis seem complex and sophisticated, some genetic analyses suggest that apomixis is inherited as a simple Mendelian trait, and it results from one or a few changes that affect the normal process of sexual reproduction [7–9].

Even though apomixis holds tremendous promise in plant breeding and seed production, the majority of apomicts do not constitute agriculturally important crops [9]. Attempts have been made to introduce apomixis into sexual crops by traditional plant breeding methods. In our previous work, the trait for apomixis was introduced from *Beta corolliflora* into the sexually reproductive species *Beta vulgaris* by traditional

Abbreviations: SSH, suppression subtractive hybridization.

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crosses of distant species. One of these hybrid lines, M14 (an apomictic monosomic addition line) was obtained (Fig. 1). Cytological studies showed that M14 contained a single chromosome of *B. corolliflora* added to the *B. vulgaris* genome, and the alien chromosome has an average transmission frequency of 96.7% through eggs [10,11]. In addition, statistical analysis of its uniformity of progeny, transmission rate and diplospory embryo sac development showed that it belonged to allium odorum type diplospory [12–14].

In recent years, progress on the physiological, developmental and molecular processes related to apomixis has been made [15–18]. However, there is little information available on the functional proteins. Whether the proteins of apomictic genes are simply not produced in sexually reproducing plants or the proteins normally functioning in sexual reproduction have altered abundance or activities, is not known [19]. To investigate the specific gene products expressed in the apomictically reproducing plants, large-scale protein expression analysis in apomictic development is needed. Recently, Zhu et al. published a small scale study of the M14 line originally obtained from our laboratory [20]. Twenty-four proteins were identified by MALDI-TOF peptide mass fingerprinting, a technique that often suffers from low reliability. In fact, eight proteins were matched with fewer than three peptides [20]. Advanced proteomics approaches, particularly high-resolution 2-DE in combination with tandem MS technology, have provided excellent tools for proteomic analysis of sugar beet seed and leaf proteomes [21,22].

In this study, we report a comparative proteomic and transcriptomic analysis of the sexual and apomictic flowers of sugar beet using 2-DE and LC-MS/MS. A total of 71 differentially expressed protein spots were successfully identified in the two samples. Of the identified proteins, only eight had corresponding transcriptomic data. This highlights the importance of expression profiling at the protein level. Taken together, this study provides new insights into the protein functions and lays a foundation for detailed studies of apomictic development.

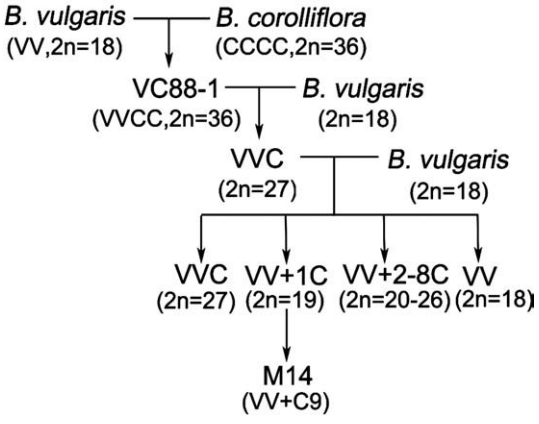

Fig. 1 – Generation of M14 apomict (*B. vulgaris* genome plus chromosome No. 9 from *B. corolliflora*).

2. Materials and methods

2.1. Materials

B. vulgaris L. and apomictic monosomic addition line M14 (containing the 18 normal chromosomes of *B. vulgaris* and chromosome 9 of *B. corolliflora*) were grown in a greenhouse of Heilongjiang University. Floral organs of apomictic M14 and sexual *B. vulgaris* were immediately frozen in liquid nitrogen and stored at −80 °C until further analysis. At least three biological replicates of apomictic M14 and three biological replicates of *B. vulgaris* were collected for 2-DE and MS analysis.

2.2. Protein extraction and quantitation

The flower samples were ground to a fine powder in liquid nitrogen. Protein was precipitated at −20 °C with 10% (w/v) trichloroacetic acid in cold acetone containing 0.07% (v/v) 2-mercaptoethanol for 2 h. After centrifuging at 20,000×*g* at 4 °C for 1 h, the precipitates were washed twice with cold acetone containing 0.07% (v/v) 2-mercaptoethanol, 1 mM PMSF and 2 mM EDTA. The pellets were lyophilized and dissolved in a 2D gel loading buffer containing 7 M urea, 2 M thiourea, 4% (w/v) CHAPS, 20 mM DTT, 2% (v/v) pharmalyte 3–10 (GE Healthcare, USA), and a Destreak reagent (GE Healthcare, USA) at room temperature for 1 h before being centrifuged at 20,000×*g* at 4 °C for 1 h. The supernatant was collected and the total protein content was quantified using a 2D gel protein quantification kit (GE Healthcare, USA).

2.3. 2-DE and image analysis

Isoelectric focusing was performed with the IPGphor system (GE Healthcare, USA). Total protein extracts (1 mg protein per sample) were loaded onto 24 cm IPG gel strips (pH 4–7), which were run at 30 V for 8 h, 50 V for 4 h, 100 V for 1 h, 300 V for 1 h, 500 V for 1 h, 1000 V for 1 h, and 8000 V for 12 h. The second dimension was run on 12.5% SDS–PAGE gels using the Ettan DALTsix Electrophoresis Unit 230 (GE Healthcare, USA) at 2 W/gel for 45 min and then at 15 W/gel for about 5 h at 16 °C. Gels were stained with Coomassie Brilliant Blue according to a protocol from Shevchenko et al. [23]. Images of the gels were scanned using an ImageScanner (GE Healthcare, USA) and analyzed using Imagemaster™ 2D Platinum software 6.0 (GE Healthcare, USA). After spot detection, the spots were matched and normalized against total spot volume using the software. The normalized volumes of the spots from replicate gels were subjected to Student's *t*-test ($p<0.05$) and only statistically significant data were considered.

2.4. Protein identification by nanoESI MS/MS analysis

Protein in-gel tryptic digestion and nanoESI MS/MS analysis were carried out on a QSTAR XL MS/MS system (Applied Biosystems/MDS Sciex, USA) as previously described [24,25]. The peptide electrospray MS/MS spectra were searched against NCBI nonredundant fasta database (8,224,370 entries, downloaded on April 14, 2009) and our EST collection (Supplemental Table 2) using MASCOT search engine (http://www.matrixscience.com). Mascot