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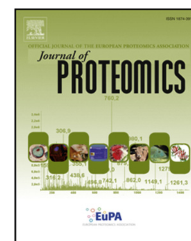

Heterosis profile of sunflower leaves: A label free proteomics approach

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ABSTRACT

Heterosis is the superior performance of heterozygous F_1-hybrid plants compared with their homozygous genetically distinct parents. The proteome of leaves of one sunflower hybrid and its parental inbred lines was analyzed by label free LC–MS/MS. A total of 1998 proteins were identified. Among them 38 proteins indicated heterosis pattern in hybrid compared with midparents. The results showed an increment of photosynthesis capacity, assimilation rate, nitrogen fixation, cell growth and reducing in some energy-consuming processes like protein production, response to stresses and respiration. These results suggest that heterosis mechanisms increase input energy of plant with reinforcement of carbon fixation pathway and reduction in consumed energy toward production of superior hybrid. This study could help to better elucidate what mechanisms are involved in heterosis of sunflower leaves and what happens at proteome level.

Biological significance
The current work describes the first study in which gel-free shotgun proteomics was used to compare the proteome of leaves of one sunflower hybrid to its parental inbred lines.
In this study 1998 proteins were identified from sunflower leaves with label free nano LC–MS/MS. The numbers of 38 proteins significantly showed heterosis pattern. The comparison between hybrid and parental inbred lines showed that hybrid vigor is actually linked by emphasizing the assimilation rate and low energy consumption.

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1. Introduction

Heterosis, or hybrid vigor, describes the superior performance of heterozygous F_1-hybrid plants in terms of increased biomass, size, yield, speed of development, fertility, resistance to disease or environmental stress compared with the average of their homozygous parental inbred lines [1,2]. Heterosis has been extensively exploited in sunflower breeding to improve productivity. Sunflower (*Helianthus annuus* L.) is an important oil crop with 48%–58% oil content [3,4]. Nowadays sunflower hybrids are cultivated as commercial varieties. Both additive and non-additive genetic effects are involved in the genetic constitution of agronomic character [5]. Significant results have been achieved in the genetic study of heterosis and the mode of inheritance of agronomically important traits, including oil content in the F_1 generation in sunflower [6–8]. Heterosis is most evident for adult traits, but it is also shown during embryo and early seedling development. Despite the rediscovery of

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heterosis about a century ago and the suggestion of various genetic models to explain this phenomenon, little consensus has been reached regarding the genetic basis of heterosis yet. Molecular mechanisms of heterosis remain to be clarified, although several hypotheses and models have been proposed, such as genome-wide dominance complementation and locus-specific overdominant effects. In addition, epistatic interactions are discussed as the main reason for the heterosis phenomenon at the molecular level [9,10]. A recent study has suggested that small-scale duplications and deletions may contribute to heterosis through complementation of deleted loci [11]. A genome-wide evaluation of cis- and trans-regulatory effects on allelic expression has also been introduced to explain molecular basis of heterosis. Hybrid vigor could also be explained by epigenetic factors. When two different genomes come together, besides obvious interactions in cis and transgenetic factors, DNA methylation machinery and its components that maintain and regulate epigenomic status would also interact [12]. Quantitative trait loci (QTL) analyses were a first step toward the molecular understanding of heterosis [13]. Several QTL [14–17] and gene expression [18–20] studies in maize and rice reported the prevalence of non-additive, while others detected mainly additive gene expression levels [21,22]. Several recent studies in different crops dissected molecular mechanism of heterosis on the genome, transcriptome and proteome level, applying a variety of molecular tools.

Protein profiling represents a key role in proteomics, enabling the comparison of protein expression across different samples or treatments. This requires sensitive and accurate assays for identifying proteins in complex mixtures and quantifying their abundances. The most commonly used approach for comparative proteomic analysis of plant tissues is the application of 2DE-gels, whereby differences in protein abundances were determined by comparing stained protein spot volumes followed by identification of proteins by mass spectrometry (MS). However, this method is limited in sensitivity, has a low dynamic range, and it is inefficient when analyzing proteins with very high or low molecular mass or for detection of highly acidic/basic, or hydrophobic proteins [23]. Gel-free shotgun proteomics is an alternative approach for the identification and quantification of proteins in large scale studies [24]. Thanks to the development of mass spectrometers with high resolution and high mass accuracy, new bioinformatics tools and computational algorithms for evaluation of quantitative differences, label-free quantification approaches have become very appealing for the quantitative analysis of biological samples. Among them spectral counting is a correlation-based method of determining relative protein quantity. This technique is based on the observed correlation between the amount of a given peptide present in a sample and the frequency with which it can be fragmented in an ion trap MS.

Liu et al. [25] have demonstrated a linear relationship between spectral counts and relative protein abundance. Thereby, they can be used as a simple and reliable index for relative protein quantification.

Proteomics is used as a main tool for gene expression in plant molecular biology. It is also used for heterosis study at molecular level. Gel-based [26,27] and gel free [28] proteomic approaches have successfully been used for detection of heterosis pattern at protein expression level in young roots or embryos of maize and rice. The result showed non-additive protein accumulation in hybrids compare with parents' average.

In this work a label-free shotgun proteomic approach was performed to study heterosis in sunflower hybrid leaves. The objective of this study was quantification of proteins with heterosis pattern in the sunflower hybrid leaves with respect to parental inbred lines. We would identify candidate proteins to provide hybrid vigor and discuss molecular mechanisms of heterosis in sunflower.

2. Material and method

2.1. Plant material

Seeds of F_1 hybrid were developed by crossing R26 male restore testers to a male sterile female inbred line, A110, with the parental inbred lines used in this study. All three genotypes were sown in triplicate completely randomized design (CRD). Plants were harvested in V8 stage [29]. All the recommended agronomic and protection practices were followed from sowing until harvesting. Three samples from each plot were harvested for measuring the dry matter. The fresh biomasses were dried in an oven at 72 °C for 48 h for dry weight determination.

2.2. Protein extraction and quantification

Proteins were extracted by TCA/acetone precipitation method developed by Damerval et al. [30], applying some modifications. Sunflower leaf tissue was frozen in liquid N_2 and ground to a fine powder using a ceramic mortar and pestle. One g of the resulting powder was suspended in 5 mL of chilled (4 °C) extraction buffer containing 175 mmol L^{-1} Tris–HCl (pH 8.8), 5% (w/v) SDS, 15% (v/v) glycerol, 25 mmol L^{-1} DTT, and 1% (v/v) plant protease inhibitor mix, and kept grinding for an additional 30 s. Homogenized cell debris were removed by filtering homogenate through two layers of miracloth, and centrifuging at low speed (500 ×*g*) at 4 °C for 15 min. The supernatant was collected and mixed by vortexing with 4 volumes of cold (−20 °C) acetone containing 10% (w/v) TCA and 0.3% (w/v) DTT, and placed at −20 °C for at least one hour for allowing protein precipitation. Then, precipitated proteins were centrifuged at 15,000 ×*g* at 4 °C for 45 min, and then the pellet was washed three times with a cold water/acetone solution (20:80, v/v) containing 0.3% (w/v) DTT and between rinses centrifuged at 15,000 ×*g* for 15 min. Successively, the supernatant was removed and the pellet was slowly dried under nitrogen and resuspended with a solution of 8 mol L^{-1} urea in 50 mmol L^{-1} NH_4HCO_3 and incubated for 1 h at 37 °C. The total protein content was determined by Bradford protein assay according to the manufacturer's instructions (Bio-Rad).

2.3. In solution digestion and off-line desalting

For each sample, protein aliquots, 100 μg (1 μg μL^{-1}), were reduced, alkylated, and digested with trypsin as described below. The disulphide bond reduction was performed with 2.5 μL of 200 mmol L^{-1} DTT, in incubation at 37 °C for 1 h,

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