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Comparative proteomic analysis of the heterosis phenomenon in papaya roots

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ABSTRACT

Heterosis describes the superior performance of a heterozygous F_1-hybrid in comparison with the average performance of the parental lines for a given trait in a given environment and is the result of the effects of non-additive genes. In the present study, proteins from the primary roots of the papaya (*Carica papaya* L.) hybrid JS12 × São Mateus and its parental inbred lines were analyzed using proteomic analyses combining the shotgun method and nanoESI-HDMSE technology. A total of 955 proteins were identified by the shotgun method, among which 261 exhibited a trend toward heterosis in the hybrid compared with the mid-parents. Non-additive proteins were divided into "above high-parent" (16.1%), "high-parent" (6.5%), "low-parent" (22.2%), and "below low-parent" (55.2%) abundance patterns. The results revealed a decrease in proteins involved in energy-consuming processes such as protein metabolism and an increase in root development proteins such as those involved in auxin polar transport and signaling regulation. The findings suggest that the hybrid possesses an optimization mechanism for protein synthesis that results in substantial improvements in cellular energy efficiency and phenotypic performance. Therefore, this study may contribute to a better understanding of the molecular basis of heterosis in papaya.

1. Introduction

Heterosis, or hybrid vigor, is a genetic phenomenon involving the superior performance of F_1-hybrid heterozygous plants in terms of increased biomass, size, yield, growth rate, fertility, disease resistance, or resistance to environmental stress compared with the average performance of their homozygous parental lines (Falconer and Mackay, 1996; Mohayeji et al., 2014). Heterosis was first described by Charles Darwin in 1876 and was independently rediscovered by George H. Shull and Edward M. East in 1908 (Hochholdinger and Hoecker, 2007). Since then, heterosis has been widely exploited in agriculture, particularly in corn, because of the large gains in productivity of the hybrids (Schnable and Springer, 2013).

Heterosis in papaya was first observed by Lassoudiére (1968) in an F_1 hybrid derived from a cross between the genotypes Philippine × Solo, with the hybrid exhibiting increased vigor and early flowering. Overall, important advances have been achieved in the development of competitive hybrids with important agronomic traits, including fruit production (Cardoso et al., 2014; Marin et al., 2006) and disease resistance (Vivas et al., 2012, 2014). For instance, the hybrid UENF/Caliman04 (UC04) shows high heterosis for key characteristics such as productivity, soluble solid content and commercial fruit quality (Cardoso et al., 2014). Because of its great economic and scientific importance, heterosis has been studied using various approaches including quantitative genetics, physiology and molecular biology. However, the regulatory mechanisms remain poorly understood due to the great complexity of this phenomenon.

Despite the major advances in genomics with respect to understanding the mechanisms responsible for the expression of characteristics (phenotypes) of agronomic interest, genomics

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alone may be insufficient to answer all questions regarding the transmission of genetic information. For instance, studies have demonstrated a substantial difference between gene expression and mRNA abundance (Schnable and Springer, 2013), processes that are constantly under the influence of different mechanisms of regulation and epigenetic control of gene expression (Banks et al., 2000). Gene expression and metabolic studies in *Zea mays*, *Oryza sativa*, and other species suggest that protein metabolism is involved in the growth differences observed between hybrids and inbreds (Goff, 2011). Thus, proteomic analysis in plants is emerging as an important tool in plant breeding because it reflects the observed variability in gene expression (Cramer et al., 2013; Eldakak et al., 2013; Pennington and Dunn, 2001).

Recent studies have applied proteomic approaches to improve our understanding of heterosis during several stages of plant development, such as in seeds (Marcon et al., 2010), during germination (Fu et al., 2011), and during root (Marcon et al., 2013) and leaf (Mohayeji et al., 2014) development. However, young roots have been considered a model for studying the molecular basis of heterosis (Hoecker et al., 2008; Yao et al., 2005). Two strategies have been employed to investigate heterosis at the protein level. One technique used to detect differences in protein abundance is two-dimensional gel electrophoresis (2-DE), which involves comparison of the size of stained protein spots followed by protein identification by mass spectrometry. Another method, the gel-free shotgun technique, implements bioinformatic tools and computational algorithms to measure quantitative differences at the protein level (Mohayeji et al., 2014), with the following advantages: increased sensitivity, identification of very high- or low-molecular-weight proteins, and detection of highly acidic, basic, or hydrophobic proteins (Domon and Aebersold, 2006; Panchaud et al., 2008).

Advances in mass spectrometry have enabled the generation of high-quality and reliable data for analyzing complex mixtures of proteins. In particular, the use of MS^E acquisition generates multiplex fragmentation data for peptides of precise mass, enabling both quantitative and qualitative characterizations of complex proteomic samples (Chakraborty et al., 2007; Silva et al., 2005).

Proteomic studies may contribute to the development of papaya, a fruit of great economic importance worldwide, and such studies to date have largely addressed responses to disease, fruit development (Angel Huerta-Ocampo et al., 2012; Rodrigues et al., 2009, 2011, 2012), and somatic embryogenesis (Vale et al., 2014). Papaya, which is grown primarily in tropical countries, is rich in nutrients, vitamins A and C, niacin and calcium (Ming et al., 2008); furthermore, various products such as papain and carpain can be extracted from this fruit (Oliveira et al., 1994).

Thus, the aim of this study was to identify and quantify differentially abundant proteins in the roots of a papaya hybrid compared with the parental lines to identify non-additive proteins that accumulate at an early stage of heterosis, to identify candidate protein biomarkers and to investigate the molecular mechanisms that promote hybrid vigor in papaya.

2. Materials and methods

2.1. Plant material

Seeds of the F_1 hybrid (UC04) (♀ JS12 × ♂ São Mateus), obtained from the crossing of different heterotic groups, and its parental lines (JS12, Formosa group and São Mateus, Solo group) were collected under similar conditions; the seed were procured from the Agricultural Caliman Company S/A, located in Linhares, Espírito Santo (ES), Brazil (19° 23′S and 40° 4′W). The seeds were disinfected for 1 min in 70% ethanol and for 10 min in 50% commercial bleach (2–2.5% sodium hypochlorite), followed by three washes with distilled, autoclaved water. The seeds were germinated in accordance with the standard protocols established by Rules for Seed Analysis (Ministeírio da Agricultura e Reforma Agraíria, 1992) using a BOD-type germination chamber set to 30 °C/20 °C (16 h light/8 h dark). Six replicates were performed; each replicate consisted of 8 Petri dishes, each containing 10 seeds, in a completely randomized design. The number of roots per seed (RN) was evaluated. Primary roots, average of 3 cm in long, were collected from all replicates of the three genotypes. For root dry matter (RDM) determination, 300 mg fresh matter (FM) was dried in an oven at 70 °C for 48 h. Samples of 300 mg FM were stored overnight at −20 °C followed by total protein extraction.

2.2. Protein extraction and quantification

Protein extracts were prepared in biological triplicate (300 mg FM each) for each evaluated genotype. Proteins were extracted using the trichloroacetic acid (TCA)/acetone precipitation method developed by Damerval et al. (1986), with modifications. This method is the most widely, reliable and efficient method for extracting proteins from plant roots (Komatsu and Hossain, 2013). Root tissue was frozen in liquid N_2 and ground to a fine powder using a ceramic mortar and pestle. The resulting powder was suspended in 1 mL chilled extraction buffer containing 10% (w/v) TCA (Sigma Chemical Co., St. Louis, MO) in acetone with 20 mM dithiothreitol (DTT) (GE Healthcare, Freiburg, Germany); the mixture was kept at −20 °C for 1 h before centrifugation at 16,000*g* for 30 min at 4 °C. The resulting pellets were washed with cold acetone plus 20 mM DTT, three times for 10 min each. The pellets were air dried, resuspended in buffer containing 7 M urea, 2 M thiourea, 2% Triton X-100, 1% DTT, 1 mM phenylmethylsulfonyl fluoride (PMSF) (Sigma-Aldrich), and 5 μM pepstatin, and incubated for 30 min on ice. The samples were then vortexed and centrifuged for 20 min. The supernatants were collected, and the protein concentrations were determined using 2-D Quant Kit (GE Healthcare, Piscataway, NJ, USA).

2.3. Protein digestion

Samples of 100 μg of total protein were prepared according to Reis et al. (2016). Initially, the samples were desalted using 5000 MWCO Vivaspin 500 membranes (GE Healthcare, Little Chalfont, UK). The membranes were filled to maximum capacity with 50 mM ammonium bicarbonate (Sigma-Aldrich) at pH 8.5 and centrifuged at 15,000*g* for 20 min at 8 °C. This procedure was repeated at least 3 times, with approximately 50 μL of sample remaining.

For protein digestion, we used the methodology described by Calderan-Rodrigues et al. (2014). Briefly, 25 μL 0.2% (v/v) RapiGest® (Waters, Milford, CT, USA) was added, and the samples were briefly vortexed and incubated in an Eppendorf Thermomixer® at 80 °C for 15 min. Then, 2.5 μL 100 mM DTT was added, and the tubes were vortexed and incubated at 60 °C for 30 min under agitation. Next, 2.5 μL of 300 mM iodoacetamide (GE Healthcare) was added, and the samples were vortexed and then incubated in the dark for 30 min at room temperature. Digestion was performed by adding 20 μL of trypsin solution (50 ng μL^{-1}) (V5111, Promega, Madison, WI, USA) and incubating the samples overnight at 37 °C. For RapiGest® precipitation, 10 μL 5% (v/v) trifluoroacetic acid (TFA, Sigma-Aldrich) was added; the samples were incubated at 37 °C for 90 min, followed by centrifugation for 30 min at 15,000*g*. The samples were then transferred to Total Recovery Vials (Waters, USA).