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Investigation of heterosis using proteomic approach

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Abstract

Heterosis is a common phenomenon in which the F_1 – hybrid of plants display superior agronomic performance compared to the inbred parental lines. For better understanding of the molecular mechanisms of plant heterosis, recently, different tissue of plant has been compared using proteomic approach between hybrid and their parental inbred lines. The patterns of plants proteome were analyzed by different software and proteins were identified by mass spectrometry/Edman sequencing method. Differential expressed proteins could be categorized in different functional classes and study of their dynamic regulation at different developmental stages could be responsible for heterosis in some plants.

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Introduction

Heterosis and proteomic approach

Heterosis or hybrid vigor was described by Shull (1952) as "the high-grade performance of an F_1-hybrid in which exhibits superior agricultural performance such as size, yield, flowering, fertility, resistance to biotic and abiotic stresses compared with its parental lines (Birchler *et al*., 2003). First of all Darwin (1987) identified this phenomenon when he was studying on F_1 maize hybrid that were taller than both parents during growth stage (Hallauer and Miranda, 1981). Scientists, in the late 1990s, estimated that maize, sorghum and sunflower formed 65% of world wild productions which were based on hybrid (Duvick, 1999). In most cases heterosis is obvious for mature traits but already confirmed during embryo and early seedling development. Depending on the models used to compare the hybrid's performance, heterosis is explained in two ways; viz: mid-parent (MPH = F1 - [(P1 + P2)/2]), and better parent (heterobeltiosis) heterosis. Mid-parent heterosis indicates that a trait shows a hybrid performance which is significantly better than the average value of the two parental inbred lines and Better-parent heterosis expresses that a hybrid trait performs significantly better than the better (Pb) of the two homozygous parental inbred lines(BPH = F1-P_b). However, from the viewpoint of breeders standard variety and better parent heterosis are more efficient (Fanseco and Peterson, 1968). Three genetic models have been explained heterosis including dominance, overdominance and epistasis (Birchler *et al*., 2003). The dominance hypothesis express that deleterious alleles or recessive mutations at different loci from the two homozygous parental lines can be complemented in the heterozygous F_1 (Fu and Dooner, 2002; Birchler *et al*., 2003). The over dominance model assumes that interaction of different alleles appear in the hybrid, leading to heterotic traits. Base on this model, it is claimed that heterizygosity alone is answerable for heterosis or hybrid vigor. Both dominance and over dominance hypotheses are based on single locus theory and are not related to molecular assumption (Shull, 1952; Gue *et al*., 2003; Birchler *et al*., 2003). Ultimately, the epistasis hypothesis is elucidated as interactions of superior alleles at different loci from two parents, and the consequences may show additively, dominance or over dominance (Yao *et al*., 2005). Hua *et al*. (2003) demonstrate that when there is a combination of two loci, epistasis is a key factor in the emergence of heterosis. The molecular mechanisms of heterosis in plant at genome-wide level have been examined by different studies, recently. These studies suggest that assessment of cis- and trans- regulatory performs on allelic expression, also has been proposed to describe molecular basis of heterosis (Hochholdinger and Hoecker, 2007; Gue *et al*., 2008; Zhang *et al*., 2008; Wei *et al*., 2009; Song *et al*., 2010). Studies between hybrid and its inbred parental line have been analyzed the modified gene expression profiling in rice, maize, wheat and Arabidopsis (Sun *et al*., 2004; Yao *et al*., 2005; Bao *et al*., 2005; Wang *et al*., 2006; Stupar *et al*., 2008; Zhang *et al*., 2008; Wei *et al*., 2009; Thiemann *et al*., 2010; Song *et al*., 2010). These investigations have been suggested that the hybridization between two inbred lines may cause to changes which are responsible for heterosis in the expression of different genes. However, other research shows that heterosis is a moderated quantitative phenotype environmentally as well epigenetic component and genome activities have potential to affect heterosis. In this way genome analysis cannot be sufficient alone (Lippman and Zamir, 2007; Barber *et al*., 2012; Chodavarapu *et al*., 2012). Although, investigation of transcriptomic analyses of gene expression remarkably have provide an understanding of molecular mechanism of heterosis in plants like rice, maize, and wheat (Yao *et al*., 2005; Zhang *et al*., 2006; Guo *et al*., 2004; Huang *et al*., 2006), which explained the transcription level of gene (mRNA), do not inevitably display the changes in the level of protein's abundance. Therefore studies are needed to settle the differential proteomes between hybrids and its parental lines and apprehend their functional roles in heterosis (Song *et al*., 2007). An important step in understanding of the gene functions is the confirmation of the protein's

existence complement in a living organism. Without a comprehensive view of protein expression, is difficult to investigate the appropriate dominant or epistatic markers from thousands of gene products. Recently proteomics has appeared as an essential methodology by a combination of several ways, e.g., separating proteins by two-dimensional electrophoresis and multiple LC, detecting proteins by MS and identifying proteins by computers and offers basal insight into function of gene that cannot be proposed by genome sequences (Canovas *et al*., 2004; Khan and Komatsu, 2004). The term of proteome was used first by Wilkines *et al*. (1995) for described whole genome protein. In fact proteomic provide the study of total expressed gene pattern by cell, tissue or special organelle under environmental conditions (Gygi *et al*., 2002). Functional proteomics investigations allow researchers to recognize the interacting between proteins and help to mapping of a protein in a particular biological pathway (Field and Song, 1989). For better understanding of heterosis by proteomic approach, we refer to the studies in this field.

Wheat root and seedling leaves proteome profile

In a study on root proteome of wheat (*Triticum aestivum* L.) between hybrid and parents 860 protein spots were displayed, by Song *et al*. (2007). Among 860 spots, 282 proteins spots from 240 different genes or gen family were identified which were grouped into 12 different classes, and primary metabolism is the most abundant group. Among 45 differentially expressed protein spots, the number of spots that displayed URH, DRH, HDH, and LDH expression pattern were 3, 3, 3 and 4, respectively. Quantitative differences was most observed and only one category that is dominant expression of uniparental genes in hybrid (UPF1), expression in hybrid of protein only expressed either in parental or maternal parent, was detected. Six protein spots expressed underdominance (DRH) or overdominance (URH). The result of this experiment is dependable with the hypothesis which multiple molecular models provide to wheat heterosis (Yao *et al*., 2005; Sun *et al*., 2004). In addition 38 of 45 differentially expressed protein spots between wheat hybrid and its parental line showed dominance, this can be determine that dominance might be the major molecular basis of root heterosis. Although, Song *et al*. (2009) studied on seedling leaves proteome in wheat (*Triticum aestivum* L.) between hybrid and parents, 900 protein spots reproducibly detected. Among these, 49 of spots were identified which being differentially expressed between hybrid and its parental line. In addition, 30 of 49 differentially expressed protein spots were pointed out which were classified in seven groups include, cell growth and division, metabolism, signal transduction, energy, disease and defense, secondary metabolism. Although comparing of protein spots pattern between hybrid and parental line, expressed both quantitative and qualitative differences. The quantitative differences can be categorized into four group: (a) up-regulated in hybrid (URH), expression in hybrid is higher than in both female and male parents; (b) down-regulated in hybrid (DRH) expression in hybrid is lower than in two parents; (c) high-dominant in hybrid (HDH), expression in hybrid is equal to the highly expressed parent; and (d) low-dominant in hybrid (LDH), expression in hybrid is equal to lowly expressed parent. In two mentioned studies, these protein spots could be observed as a representation of part of the expressed proteins in the hybrids and their parents. Thus the results obtained from differentially expressed proteins of seedling leaves and roots represent the level and pattern of these proteins spots between wheat hybrids and their parents.

Rice proteome profile

Wang *et al*. (2008) studied the profile proteome of rice embryos from a hybrid cultivar and its parental line. The 326 protein spots were observed in three rice line and 54 differentially expressed proteins spots were identified in major biological stages including nutrient reservoir, response to stress, and metabolism. The assignment of the protein spots classified into three general group: additivity, overdominance, and underdominance. Most of the storage proteins showed overdominance pattern and

proteins of stress category display additivity. Although the result of proteomic was compared with transcriptomic data and 28 candidate heterosis-associated genes were discovered.

Zhang *et al.* (2012) analyzed the reveals dynamic proteome changes between superhybrid rice LYP9 and its parental line at three developmental stages including: tillering, flowering, and grain-filling. Totally 384 differentially expressed proteins spots (DP) were discovered and 297 DP were identified, corresponding to 222 unique proteins. All identified DP were grouped into 14 categories. DP was segregating into two groups those between the parents (DP_{PP}) and between the hybrid and its parents (DP_{HP}). The result of comparing these indicated that proteins in three groups were mainly improved including: photosynthesis, glycolysis, and disease/defense. Furthermore, in comparison of flowering and grain-filling stages with tillering one, showed that the number of identified DO_{HP} involved in photosynthesis, glycolysis, and disease/defense increased at two first mentioned stages. The up-regulated DP_{HP} involved in the three groups displayed more extensive expression in LYP9 at flowering and grain-filling stages than tillering stage. As well, at flowering and grain-filling stages LYP9 expressed high increase of the rate of Co_2 assimilation and discernible quantum yield of photosynthesis than tillering stage. These results propose that the proteins involved in three highest categories as well as their dynamic regulation at different developmental stages can be responsible for heterosis in rice.

Maize proteome profile

Hoecker *et al.* (2008) analyzed the noadditive protein accumulation in young primary roots of maize (*Zea mays* L.) F_1-hybrid in comparison with its parental line UH002 and UH301. Studies has been indicated that heterosis during early root development is exhibited that the young root system is an acceptable model for molecular studies of the early stages of heterosis (Hoecker *et al.*, 2006). In this study the most abundant soluble proteins investigated in 3.5-day-old primary roots before the phenotypic expression of heterosis in inbred lines and corresponding hybrid UH301 × UH002. In 2-DE gels, 304 proteins spots were expressed which among them 150 proteins spots were detected. These proteins were accumulated in a nonadditive class in the hybrid compared to the average of their inbred parental lines. Expression of 51% of the nonadditively accumulated proteins was more than hybrid or below the low parent. Analysis by ESI-MS/MS method identified that 75 of the 76 proteins categorized to these expression classes. Of 75 proteins, the most abundant functional classes were belonged to metabolism and disease and defense which were encoded by 60 different genes. Nonadditive protein accumulation in primary roots of maze hybrids could be related and associated with heterosis manifestation.

Fu *et al.* (2011) studied the proteome of maize seed during germination to analysis the heterosis. Totally, 257, 363, 351, 242, and 244 nonadditively expressed proteins were identified in five hybrid cultivar of maize embryos, from germinating seeds after 24h of soaking. These nonadditive proteins were classified into six group including: above high-parent, high-parent, partial dominance, low-parent, below low-parent, and D expression pattern. Most of the nonadditive proteins were stored in the high-parent (21 proteins) and above high-parent (23 proteins) expression pattern. Five proteins spots expressed low-parent expression, three displayed below low-parent. The observed patterns revealed the important roles of dominance, partial dominance, and over dominance during mentioned seed germinating of maize. In addition, by using mass spectrometry method, 54 different proteins were identified and classified into nine functional group: metabolism (9), cell detoxification (8), unknown functional proteins (8), chaperons (7), signal transduction (6), development process (5), other (5), transporter (3), and stress response (3). Moreover a nonadditive expression pattern, including partial dominance, dominance and overdominance, is the major

exhibition of heterosis during maize germination. Despite proteins of chaperones, signal transduction, and cell detoxification may perform in seed germination and heterosis configuration during uptake water in phase II of germination which their importance on degree of heterosis is different with differential accumulated expression patterns for each hybrid.

Marcon *et al.* (2010) studied heterosis of maize (*Zea mays* L.) in embryos during development. F1-hybrids include UH005 × UH250 and UH250 × UH005 were analyzed by two-dimensional electrophoresis and surveyed the most abundant proteins of hybrids and their parental inbred lines 25 and 35 days after pollination. Totally 597 differentially expressed proteins spots were identified which 141 proteins spots revealed nonadditive accumulation levels among them in at least one hybrid. The largest number of nonadditively expressed proteins classified as low parent. Nonadditively accumulated proteins were classified based on their relative expression levels according to the stupar and springer (2006) classification. Most of nonadditively expressed proteins (66%) exhibited the levels of accumulation similar to or between the parental inbred line values. The 44% proteins showed below low parent levels or above of better parent and low parent value. Analysis of 141 proteins by mass spectrometry revealed that development, protein metabolism, redox-regulation, glycolysis, and amino acid metabolism were the most important functional classes among nonadditively accumulated proteins. In embryos of the UH250 × UH005 hybrid, were observed a significant up-regulation of enzymes related to glucose metabolism which was more than the best parent. Totally 193 nonadditive expression patterns were observed in four genotype/stage association analyzed which among this 52 proteins were identified in 25-day-old hybrids embryos but the most of nonadditively accumulated proteins were related with 35-day-old hybrids.

In another study, Macron *et al.* (2013) analyzed the heterosis in proteome of maize (*Zea mays* L.) seminal roots by quantitative label-free LC-MS. Two cultivar of hybrids and their parental inbred lines were studied and 1918 proteins were detected by label-free LC-MS/MS totally. Eighty-five proteins expressed nonadditive accumulation in at least one hybrid. The most abundant class of nonadditive proteins that represented by 27 proteins, related to functional category protein metabolism. Inside this group, 16 of 17 nonadditively accumulated ribosomal proteins revealed high or above high parent expression in seminal roots. The comparison of common hybrids proteome and their parental lines manifests an increased protein synthesis rate in hybrids which can present to the early manifestation of heterosis in seminal roots.

Comparative proteomic analysis between maize hybrid and its parental inbred lines of embryos during early stages of seed germination were done by Gue *et al.* (2013). Totally, 1140 and 1443 proteins spots were observed which among them 134 and 191 proteins spots showed differentially expressed between hybrids and its parental lines, respectively. These data implied that significant changes in protein level occurred in hybrids in comparison with their parents, could correlate with observed heterosis. In addition, 54.55% of nonadditively accumulated proteins in 24h imbibed seed embryos expressed above or equal to the higher parent patterns level. Furthermore, 155 of proteins spots were identified which were categorized into eight functional classes including: transcription/translation, energy /metabolism, signal transduction, disease/defense, storage protein, transposable element, cell growth and division and unclassified proteins. The largest group in dry and 24 h imbibed embryos is transcription/translation (22% and 30%) energy/metabolism (16% and 19%), disease/defense (15% and 13%) and storage protein (10% and 17%). Additional studies revealed that 155 protein spots which identified derived from 118 different genes or gene families.

Conclusion

Proteomic technique may be useful for investigating the molecular mechanism of heterosis at the translational level and several studies is indicated its use in exploring post-transcriptional protein differences between hybrids and their parents (Hoecker *et al*., 2008a, 2008b; Song *et al*., 2007; Muthreich *et al*., 2010). For the measured traits, the heterosis performance, is restricted by environmental quantitative phenotype because of that most of analysis cannot incorporated into proffered heterotic mechanisms. So then it is better to focus on a simple trait which is influenced by environmental variability, hardly or by another traits to survey the molecular level of heterosis (Lippman and Zamir, 2006).These results suggested that hybridization between two parental lines can cause change in expression level of a diversity of proteins and gene expression of translational level in the hybrid may be responsible for the detected heterosis (Gue *et al*., 2013).

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