Scientia Horticulturae 169 (2014) 214–225

Contents lists available at ScienceDirect

Scientia Horticulturae

journal homepage: www.elsevier.com/locate/scihorti

Gene expression profiling and identification of resistance genes to low temperature in leaves of peanut (*Arachis hypogaea* L.)

Na Chen[a], Qingli Yang[a], Dongqing Hu[b], Lijuan Pan[a], Xiaoyuan Chi[a], Mingna Chen[a], Zhen Yang[a], Tong Wang[a], Mian Wang[a], Yanan He[a], Shanlin Yu[a,*]

[a] *Shandong Peanut Research Institute, Qingdao 266100, PR China*
[b] *Qingdao Entry-Exit Inspection and Quarantine Bureau, Qingdao 266001, PR China*

ARTICLE INFO

Article history:
Received 3 May 2013
Received in revised form 24 January 2014
Accepted 25 January 2014
Available online 16 March 2014

Keywords:
Gene expression profiling
Microarray
Cold stress
Peanut (*Arachis hypogaea* L.)

ABSTRACT

The cultivated peanut (*Arachis hypogaea* L.) is important oil crop and cold stress seriously influences its development and yields. Tolerant varieties produced using transgenic techniques will effectively increase peanut plantation area and enhance yields. However, little is known about the network of gene expression regulation related to cold stress in peanut. Therefore, we screened genes regulated by cold stress in peanut. In total, 1478 up-regulated and 1510 down-regulated probe sets were successfully identified in leaves of peanut subjected to cold stress using the microarray technique. Data analysis indicated that the main biological processes involved in cold stress response included signal transduction, transcript regulations and accumulation of soluble metabolites. Regulation of cell division, translation, protein modification, transport and DNA/RNA-related processes were also involved in peanut cold acclimation. Our study also revealed that protein kinases, transcription factors, heat shock proteins, resistance proteins, stilbene synthase, and so on, may play important functions in cold stress regulation of peanut. The function of some probe sets in cold stress regulation had not been clarified (e.g. proteins functioning in glycerol ether metabolic process, microtubule-based movement or nutrient reservoir activity). Some genes we identified lack functional annotation and their roles in response to cold are yet to be elucidated. These results showed an overview of the transcription map of peanut under cold stress, which may yield some useful insights into cold-mediated signal transduction pathways in peanut and offer some candidate genes as potential markers of tolerance to cold stress.

1. Introduction

Plant growth and yield are strongly influenced by abiotic stresses such as drought, salt and cold. Plants respond and adapt to these conditions with an array of biochemical and physiological changes (Hsieh et al., 2002; Zhu et al., 2007). Many adaptation processes are regulated by stress-responsive gene expression. Plants respond with changes in gene expression and protein products when exposed to low temperatures. Such ability has an impact on the plant distribution, survival and crop yields. Conventional breeding methods have met with limited success in improving the cold tolerance of important crop plants (Sanghera et al., 2011). Recent studies involving full-genome profiling/sequencing, mutational and transgenic plant analyses, have provided a deep insight into the complex transcriptional mechanism of plants under cold stress. The alterations in expression of genes in response to low temperature are followed by increases in the levels of hundreds of metabolites which play protective roles against the damage of cold stress. A number of genes that are responsive to cold stress have been isolated and characterized. Many studies have indicated that cold-regulated gene expression is critical in plants for both chilling tolerance and cold acclimation (Sanghera et al., 2011).

Key to the tolerance of plants to abiotic stresses is a complex network of transcription factors and other regulatory genes that control multiple defense enzymes, proteins and pathways (Cushman and Bohnert, 2000). Transcriptome analysis using microarray technology is a powerful technique, shown to be very useful for discovering stress-inducible genes involved in stress

Abbreviations: DEGs, Differentially expressed genes; ds-cDNA, Double- stranded cDNA; RMA, Robust multichip analysis; SAM, Significance analysis of microarrays; FC, Fold change; FDR, False discovery rate; GO, Gene ontology.

* Corresponding author. Tel.: +86 532 87626830; fax: +86 532 87626832.
E-mail addresses: chenna7948@163.com (N. Chen), rice407@163.com (Q. Yang), Dongqinghu@163.com (D. Hu), panlijuan_2008@yahoo.com.cn (L. Pan), xiaoyuanchi@yahoo.com.cn (X. Chi), chenmn04@126.com (M. Chen), woozhyang@hotmail.com (Z. Yang), wtwtp@126.com (T. Wang), wmshmilyzcx@126.com (M. Wang), yananhe08@yahoo.com.cn (Y. He), shanlinyu2012@163.com, yshanlin1956@163.com (S. Yu).

http://dx.doi.org/10.1016/j.scienta.2014.01.043
0304-4238/

response and tolerance (Shinozaki et al., 2003; Seki et al., 2004; Zhao et al., 2008). Zhao et al. (2008) identified 242 unique genes expressed differentially between cold-sensitive and -tolerant rice using DNA chips. These genes are involved in processes such as senescence, cell death, male sterility and plant hormone response. Similarly, global transcript profiling analyses indicated that >10% of genes in the *Arabidopsis thaliana* genome were regulated during cold acclimation (Kreps et al., 2002; Seki et al., 2002, 2004).

A cis-acting element, dehydration responsive element (DRE) identified in *Arabidopsis*, is involved in ABA-independent gene expression under abiotic stresses such as drought, cold and salt. The overexpression of some drought responsive transcription factors can lead to the expression of downstream genes and enhanced abiotic stress tolerance in plants. These gene products are classified into two groups (Kreps et al., 2002; Seki et al., 2002). The first group of proteins includes late embryogenesis abundant (LEA) proteins, chaperones, osmotin, antifreeze proteins, mRNA-binding proteins, some key enzymes for osmolyte biosynthesis (such as water channel proteins, proline, sugar and proline transporters, and detoxification enzymes), enzymes for fatty acid metabolism and lipid-transfer proteins (Holmberg and Bülow, 1998). Some of these stress-inducible genes – especially encoding proteins such as enzymes for osmolyte biosynthesis, LEA proteins and detoxification enzymes – have been reported as overexpressed in transgenic plants and produced stress-tolerant phenotypes (Cushman and Bohnert 2000; Shinozaki et al., 2003). The second group contains protein factors involved in regulation of signal transduction and gene expression that probably function during stress response (Seki et al., 2003). This group includes various kinases and transcription factors that regulate different stress-inducible genes collectively or separately, and may constitute gene networks. Seki et al. (2003) reported that some of these regulatory pathways are also involved in cold stress responses. Although the clear functions of most of these genes are not fully understood, functional analysis of these stress-inducible transcription factors will provide precise information on the complex regulatory gene networks involved in responses to abiotic stresses (Tester and Bacic, 2005; Zhao et al., 2008). Some of these stress-inducible regulatory genes that encode transcription factors have been overexpressed in transgenic plants and generated stress tolerant phenotypes (Zhang et al., 2004; Vinocur and Altman, 2005).

The existence of *CBF*-independent pathways is also supported by analysis of mutants with increased freezing tolerance. For example, mutations in eskimo1 (*esk1*), a protein of unknown function, result in constitutive freezing tolerance (Xin and Browse, 1998; Bouchabke-Coussa et al., 2008). Transcriptome comparison of *CBF2*-overexpressing plants and *esk1* mutants showed that different sets of genes are regulated by CBF2 and ESK1 (Xin et al., 2007). Hos9 (a homeodomain protein) is a transcription factor necessary for cold tolerance in *Arabidopsis* (Zhu et al., 2004). Transcriptome analysis revealed different CBF and HOS9 regulons (Zhu et al., 2004). Some members of the abiotic, plant hormone and pathogen-inducible ERF family also play a crucial role in cold stress such as *CaPF1* (Yi et al., 2004) and *TaERF1* (Xu et al., 2007). These results suggested that several transcriptional networks operate during cold acclimation and cold stress tolerance of plants.

Posttranscriptional regulation such as pre-mRNA processing, mRNA stability and mRNA export from the nucleus also play important functions in cold tolerance of plants (Chinnusamy et al., 2007).

The cultivated peanut (*Arachis hypogaea* L.) is an important oil crop grown extensively in tropical, subtropical and temperate climates. The peanut plant needs relative higher temperature throughout the whole development process, and cold stress seriously influences its germination, development, bloom and yields (Wang et al., 1985; Feng 1991; Upadhyaya et al., 2001). Transgenic techniques are effective tools to enhance cold-stress tolerance, and tolerant varieties produced using transgenic techniques will effectively increase peanut plantation area and enhance yields. However, little is known about the network of gene expression regulation related to cold stress in peanut except several genes were proved to be cold regulated (Dave and Mitra, 1998, 2000; Rudrabhatla and Rajasekharan, 2002). Therefore, we used a microarray that included 36,158 peanut unigene ESTs to monitor the expression profiles of genes related to cold stress regulation in peanut. The biological processes involved in the response of peanut to cold stress were studied using Gene Ontology (GO) enrichment analysis for the up- or down-regulated genes. This article is an overview of the transcription map of peanut under cold stress, which may yield some useful insights into cold-mediated signal transduction pathways in peanut and offer a number of candidate genes as potential markers of tolerance to cold stress.

2. Materials and methods

2.1. Plant material and treatments

The plant material used in this study was peanut cultivar Huayu 19, which had been proved to be relatively cold torlerant in our previous study (data not shown). Peanut seeds were germinated in a mixture of nutrient-rich soil and vermiculite (2:1) and grown under conditions of 16/8 h and 28/22 °C of light/dark.

The cold treatment was applied according to the studies reported previously in *Arabidopsis* and rice (Lee et al., 2005; Dai et al., 2007). Seedlings at the trefoil leaf stage were maintained at 4 °C in a light incubator. The leaves were collected after 48 h of cold treatment and untreated leaves were used as controls. All the samples were immediately frozen in liquid nitrogen and then stored at −80 °C until required.

2.2. RNA isolation

Total RNA was isolated from samples using the RNeasy Mini Kit (Qiagen) according to the manufacturer's instructions. RNA samples were purified with a NucleoSpin® RNA clean-up kit (MACHEREY-NAGEL, Germany). RNA quality was assessed by formaldehyde agarose gel electrophoresis and was quantified spectrophotometrically. All RNA was stored at –80 °C until further analysis.

2.3. Microarray construction and hybridization procedure

A custom long-oligo nucleotide(60-mer) array (12 × 135 k) was manufactured based on NimbleGen proprietary Maskless Array Synthesizer (MAS) technology in CapitalBio Corporation (Beijing, China). Microarrays contained 36,158 probe sets.

Total RNA of 5 μg was used to synthesize double-stranded cDNA (ds-cDNA) using Invitrogen SuperScript ds-cDNA synthesis kit. Then the ds-cDNA was cleaned and fluorescently labeled using Klenow enzyme in accordance with the Nimblegen Gene Expression Analysis protocol (Nimblegen Systems, Inc., Madison, WI, USA). Microarrays were hybridized, stained and processed at CapitalBio Corporation (Beijing, China) using Roche (Shanghai, China) NimbleGen systems as previously described (Yu et al., 2011). The experiment was performed three times, resulting in three biological replications for both control and cold treatment samples.

2.4. Data analysis

After hybridizations, microarrays were scanned using the Roche NimbleGen MS200 scanner and the data of obtained images were extracted with NimbleScan software at CapitalBio Corporation.