Plant Physiology and Biochemistry 105 (2016) 21–28

Contents lists available at ScienceDirect

Plant Physiology and Biochemistry

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

Research article

Proteomics analysis of compatibility and incompatibility in grafted cucumber seedlings

Qing Xu [a], Shi-Rong Guo [a], Lin Li [a], Ya-Hong An [a], Sheng Shu [a], Jin Sun [a, b, *]

[a] College of Horticulture, Nanjing Agricultural University, Key Laboratory of Southern Vegetables Genetic Improvement, Ministry of Agriculture, Nanjing, 210095, PR China
[b] Institute of Facility Horticulture, Nanjing Agricultural University, Suqian, 223800, Jiangsu Province, PR China

ARTICLE INFO

Article history:
Received 21 September 2015
Received in revised form
29 March 2016
Accepted 1 April 2016
Available online 2 April 2016

Keywords:
Graft compatibility
Cucumber
Pumpkin rootstock
Proteomics

ABSTRACT

Graft compatibility between rootstock and scion is the most important factor influencing the survival of grafted plants. In this study, we used two-dimensional gel electrophoresis (2-DE) and matrix-assisted laser desorption/ionization tandem time-of-flight mass spectrometry (MALDI-TOF/TOF MS) to investigate differences in leaf proteomes of graft-compatible and graft-incompatible cucumber (*Cucumis sativus* L.)/pumpkin (*Cucurbita* L.) combinations. Cucumber seedlings were used as the scions and two pumpkin cultivars with strongly contrasting grafting compatibilities were used as the rootstocks. Non-grafted and self-grafted cucumber seedlings served as control groups. An average of approximately 500 detectable spots were observed on each 2-DE gel. A total of 50 proteins were differentially expressed in response to self-grafting, compatible-rootstock grafting, and incompatible-rootstock grafting and were all successfully identified by MALDI-TOF/TOF MS. The regulation of Calvin cycle, photosynthetic apparatus, glycolytic pathway, energy metabolism, protein biosynthesis and degradation, and reactive oxygen metabolism will probably contribute to intensify the biomass and photosynthetic capacity in graft-compatible combinations. The improved physiological and growth characteristics of compatible-rootstock grafting plants are the result of the higher expressions of proteins involved in photosynthesis, carbohydrate and energy metabolism, and protein metabolism. At the same time, the compatible-rootstock grafting regulation of stress defense, amino acid metabolism, and other metabolic functions also plays important roles in improvement of plant growth.

1. Introduction

Grafting is a technique in which a seedling shoot or stem—the scion—is attached to the appropriate point of another seedling known as the rootstock, thereby merging both seedlings into a single new plant. This method plays an important role in plant propagation and improvement. Because grafting may improve growth performance and fruit production and quality by enhancing photosynthetic capacity, root nutrient absorption, disease resistance, and stress tolerance of the graft combination (Martínez-Ballesta et al., 2010), this approach is widely used for horticultural production. The effectiveness of grafting primarily depends on survival rate and the symbiotic ability of rootstock and scion throughout the subsequent growth period. A variety of factors affect the survival of grafted plants, including graft compatibility between rootstock and scion, the grafting technique used, and external environmental conditions during the wound healing process (Leonardi and Romano, 2004). Graft compatibility between rootstock and scion is obviously the most important of these factors which is defined as the capacity to undergo normal graft-union healing, growth, flowering and fruit bearing after grafting between rootstock and scion and required for successful symbiosis between the two joined plants. Graft incompatibility may occur as a result of genetic and structural differences between rootstock and scion, a phenomenon directly restricting application of the grafting technique in horticultural production. According to Davis et al. (2008), graft incompatibility may cause grafted plants to lose nutrients and water, resulting in plant wilting, and it might even reduce fruit production and quality in a more severe circumstance. Because graft compatibility is extremely important, elucidation of the underlying molecular mechanism is very worthwhile.

* Corresponding author. College of Horticulture, Nanjing Agricultural University, Nanjing, 210095, PR China.
E-mail address: jinsun@njau.edu.cn (J. Sun).

http://dx.doi.org/10.1016/j.plaphy.2016.04.001
0981-9428/

An increasing number of studies on graft compatibility have appeared in recent years. Most of this research has focused on aspects of physiological biochemistry, such as defense-enzyme activity, hormonal regulation, and morphological structure (Ermel et al., 1997; Aloni et al., 2010). In incompatible grafting melon combinations, superoxide dismutase and peroxidase activities have been found to be lowered while reactive oxygen species (ROS) levels are increased (Aloni et al., 2008). Phenylalanine ammonia lyase (PAL), an important enzyme in phenylpropanoid metabolism, is responsible for the biosynthesis of many secondary metabolites. According to Pereira et al. (2014), the PAL activity of some incompatible *Prunus* spp. combinations is higher than that of their compatible counterparts. Errea (1998) has also demonstrated a link between graft incompatibility and phenolic compound accumulation in fruit trees. In our previous study on physiological differences between graft-compatible and the opposite combinations, we observed stronger resistance to grafting-induced oxidative stress and weaker phenylpropanoid metabolism in the former and lower chlorophyll fluorescence levels in the latter (Xu et al., 2015). In spite of these findings, only a limited number of studies have investigated the precise molecular biological mechanism of graft compatibility.

Proteomics is a powerful tool for describing how the proteome is affected by different physiological conditions. To obtain a comprehensive and deeper understanding of all kinds of complex life activities, the use of proteomics analysis, a new research field focusing on the entire set of proteins in a cell or a tissue of an organism, is essential. Yang et al. (2012) applied 2-dimensional gel electrophoresis (2-DE) coupled with matrix-assisted laser desorption/ionization tandem time-of-flight mass spectrometry (MALDI-TOF/TOF-MS) techniques and found that the salt tolerance of rootstock-grafted watermelon would contributed to the higher expressions of key enzymes involved in the metabolic systems includes Calvin cycle, glycolic pathway, amino acids biosynthesis, and the TCA cycle. Recently, Vitale et al. (2014) used a proteomic approach and highlighted the accumulation of specific proteins which seem to highly contribute in eliciting resistance to pathogen. However there are no reports about effects of graft compatibility on cucumber seedlings proteomics at the moment.

Cucumber (*Cucumis sativus* L.) is one of the main vegetables grown under protected cultivation in China. According to United Nations Food and Agriculture Organization statistics FAO (http://faostat.fao.org), the area devoted to cucumber cultivation in China has increased annually since 1997 and currently ranks number one in the world. In 2013, the area of Chinese cucumber cultivation encompassed 1,166,690 hm^2 and total production was 54,362,750 tons. Despite this importance, cucumber plants are characterized by low resistance to various stress conditions because of their fragile roots and sensitivity to unsuitable cultivation conditions. Grafting has been shown to enhance cucumber plant stress resistance and to increase production under various cultivation systems; moreover, grafting technology has been widely applied to cucumber seedling production and yielded tremendous cultivation benefits. Nevertheless, many problems are currently associated with cucumber grafting practices, with graft incompatibility one of the major limiting factors. In this study, we therefore explored protein expression differences in cucumber leaves of graft-compatible and graft-incompatible combinations. We used cucumber seedlings as scions, two pumpkin (*Cucurbita* L.) cultivars with large differences in grafting compatibility as rootstocks, and non-grafted and self-grafted cucumber seedlings as control groups. Through proteomics, our study aimed to provide insights to facilitate a better understanding of graft compatibility in cucumber seedlings.

2. Materials and methods

2.1. Plant materials and union establishment

Seedlings of cucumber 'Jinchun No. 4' (Tian Kerun Cucumber Institute, Tianjin, China) were used as scions, while seedlings of compatible pumpkin cultivar 'Heizinangua' (*Cucurbita ficifolia* Bouche; Hongwei Seed Co., Shouguang, China), and incompatible pumpkin cultivar 'Dongyangshenli' (*Cucurbita moschata* D.; Fengyuan Seed Co., Shouguang, China) were used as rootstocks (Xu et al., 2015). There were four test set treatments: (1) graft-compatible cucumber/'Heizinangua' combinations (C); (2) graft-incompatible cucumber/'Dongyangshenli' combinations (I); (3) self-grafted (SG) and (4) non-grafted (NG) cucumber seedlings were used as controls. Seeds of rootstocks were sown in 15-cell polystyrene trays filled with commercial organic substrate (2:2:1 [v/v/v] vinegar waste compost:peat:vermiculite; Beilei, Zhenjiang, China), and scion seeds were sown in 72-cell trays when the rootstock seeds had just emerged. When the cotyledons of scions and first true leaves of rootstocks had fully opened, hole insertion grafting was performed. The growing point of each rootstock was removed with a sharp probe and a hole was punched in the upper portion of its hypocotyl. The scions were removed from the tray, cut at 35°–45° angles on both sides of the hypocotyl, and inserted into the holes in the rootstocks (Fig. 1). The seedlings were arranged in a completely randomized design with three replicates per treatment, and four 15-cell polystyrene trays per replication with 60 seedlings. All grafting was performed by one operator.

Grafted plants were transferred to a small plastic arched shed and maintained at a temperature above 25 °C and a relative humidity between 85% and 100% for approximately 7 days until the graft union had healed. Twenty-five days after grafting, fully expanded third leaves were harvested, immediately frozen in liquid nitrogen, and stored at −80 °C until processing for protein extraction.

2.2. Protein extraction

Protein extraction was performed using a modified version of the trichloroacetic acid-acetone precipitation method described by Hurkman and Tanaka (1986). Leaf samples (1–2 g fresh weight) were ground in a mortar with liquid nitrogen. Frozen leaf tissue was finely ground in liquid nitrogen and homogenized in ice-cold

Fig. 1. Graft union produced by hole insertion grafting in the graft-compatible combination.