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Protein sHSP26 improves chloroplast performance under heat stress by interacting with specific chloroplast proteins in maize (*Zea mays*)

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ABSTRACT

We recently demonstrated that chloroplast small HSP26 (sHSP26) is abundant in maize leaves under heat stress and potentially involved in maize heat tolerance. However, it largely remains unclear how sHSP26 functions in maize under heat stress. Here, 2-DE-based proteomics, RNA interference (RNAi), co-immunoprecipitation (Co-IP) and yeast two-hybrid (Y2H) were used to reveal chloroplast proteins interacting with sHSP26 and how sHSP26 functions under heat stress. After the silencing of *sHSP26*, a total of 45 protein spots from isolated protoplasts were greatly changed in abundance, of which 33 spots are chloroplastic. Co-IP revealed that nine proteins possibly associated with sHSP26. Y2H demonstrated that six chloroplast proteins interact with sHSP26 under heat stress. In particular, four proteins, including ATP synthase subunit β, chlorophyll a–b binding protein, oxygen-evolving enhancer protein 1 and photosystem I reaction center subunit IV, strongly interacted with sHSP26 and their abundance greatly declined after RNAi of sHSP26 under heat stress. In addition, H_2O_2 accumulation in the chloroplasts significantly increased the expression of sHSP26, and the suppression of sHSP26 expression significantly reduced the O_2 evolution rate of photosystem II under heat stress. Overall, these findings demonstrate the relevance of sHSP26 in protecting maize chloroplasts under heat stress.

Biological significance

Maize is one of the most important crops worldwide. Frequent heat stress reduces significantly the yield and quality of maize. Our results demonstrated that sHSP26 improved maize chloroplast performance under heat stress by interacting with specific proteins. These findings are useful for understanding the mechanism of heat stress response and heat-tolerant molecular breeding in maize.

Abbreviations: Co-IP, co-immunoprecipitation; OEC, oxygen evolving complex; PS II, photosystem II; PEG, polyethylene glycol; PQ, paraquat; RNAi, RNA interference; Y2H, yeast two-hybrid.

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1. Introduction

Heat stress is one of the most common abiotic stresses for many crops worldwide and reduces the yield and quality of crops. The average global temperature increase was approximately 1 °C in the last decade [1]. Thus, there is a need to increase crop productivity with increasing temperatures worldwide. It was recently shown that the heat tolerance of plants can be enhanced through a transgenic approach using small sHSPs (sHSP26) [2]. Plant sHSPs are nuclear-encoded and vary in size from 15 to 43 kDa, are produced ubiquitously in response to heat stress and play important roles in the heat tolerance of plants [3,4].

Chloroplast-localized sHSPs usually have three highly conserved regions named consensus I, II, and III. Two conserved regions are present in all small heat shock proteins, whereas region III (a methionine-rich region) is specific to chloroplastic sHSPs [5]. Photosystem II (PS II) is one of the most labile protein complexes that are affected by abiotic stress [6]. sHSPs function in the protection of PS II during heat stress [2,7] and cold stress [8]. The chloroplast is one of the major sites for the generation of ROS during normal photosynthetic electron transfer, and the increase in ROS generation during stress adversely affects several macromolecules. There is emerging evidence that oxidative stress increases the expression of sHSP genes [7,9]. Conceivably, sHSP26 might be involved in oxidative stress tolerance. The unique methionine-rich amphipathic α-helix structure of sHSP26 and their ability to undergo oxidation-dependent conformational changes may protect plants from oxidative stress [9,10]. The sHSP26 of *Chenopodium album*, which is localized in the thylakoid lumen, interacts specifically with the thermolabile oxygen-evolving complex of PS II [7,11].

sHSP26 is expressed in many plant species following oxidative or heat stress and protects the photochemical efficiency of PS II against oxidative and heat stress [2,7,9]. We previously showed that sHSP26 is highly inducible by heat stress in maize seedling leaves [12]. However, it largely remains unclear how sHSP26 functions under heat stress. In this study, 2-DE based proteomics, RNA interference (RNAi), co-immunoprecipitation (Co-IP) and yeast two-hybrid (Y2H) approaches were used to determine the specific chloroplast proteins interacting with sHSP26 and the mechanisms by which sHSP26 provides maize tolerance to heat stress.

2. Materials and methods

2.1. Material and treatments

All of the reagents were purchased from Sigma-Aldrich unless otherwise stated. Maize seeds (*Zea mays* L. cv. Zhengdan 958) were used in the experiments. Currently, Zhengdan 958 is the most widely cultivated high-yield maize hybrid in China. The seeds were surface-sterilized for 10 min in 2% (v/v) hypochlorite and germinated on moistened filter paper. The maize seedlings were grown in Hoagland's nutrient solution with 400 μmol m^{-2} s^{-1} photosynthetic active radiation, a 14/10-h day/night cycle, a day/night temperature of 28/22 °C, and a relative humidity of 75% in a light chamber. When the second leaves were fully expanded, the seedlings were subjected to various treatments.

Drought stress was imposed by placing the seedlings in a PEG solution (−0.7 MPa) for 8 h at 28 °C and 40% relative humidity. Heat shock was applied for a total of 8 h by increasing the temperature from 28 to 42 °C at a rate of 2 °C h^{-1} and then maintaining the temperature at 42 °C for 1 h. Accordingly, each stress treatment had a duration of 8 h. The control seedlings were maintained at 28 °C and 75% relative humidity.

To study the effects of H_2O_2 scavengers, the plants were pretreated with 10 mM KI for 8 h and then exposed to heat stress for 8 h as described above. For the oxidative stress treatment, the plants were incubated with 10 μM paraquat (PQ) for 8 h as described above. The expanding leaves (second from the top) of the treated and untreated seedlings were sampled, frozen immediately in liquid nitrogen, and stored at −80 °C until analysis.

2.2. Western blotting

Maize leaf samples were homogenized in an SDS-containing buffer (1:3 g/ml, 2% (w/v) SDS, 100 mM Tris–HCl, pH 6.8, 20% sucrose and 2% DTT) using a mortar and pestle. The supernatant that was obtained after centrifugation (15,000 *g*, 10 min) was separated on a 12.5% SDS-PAGE gel. After electrophoresis, the proteins were transferred onto a polyvinylidene difluoride membrane and probed with a monoclonal antibody that was raised against maize sHSP26 [13]. To standardize the results, the relative abundance of β-actin was also determined and used as an internal standard.

2.3. Protoplast isolation

Maize mesophyll protoplasts were isolated using the method of Sheen [14]. The cell wall was digested in an enzyme solution containing 1.5% (w/v) cellulose R10 (Yakult Honsha, Tokyo, Japan) and 0.2% (w/v) macerozyme R10 (Yakult Honsha, Tokyo, Japan). The isolated protoplasts were washed twice with the incubation buffer (0.5 M mannitol, 2 mM MES, pH 5.7, 20 mM KCl) before protein extraction and dsRNA transfection.

In the protoplast experiment, the protoplasts were transfected with dsRNA against maize sHSP26 (dsHSP26) or with water (no dsHSP26) and incubated for 12 h. The protoplasts were then incubated at 42 °C for 30 min under heat stress or for 10 min after pretreatment with 1 μM PQ to determine the O_2 evolution rate of PS II electron transport.

2.4. Protein extraction

The protoplasts were centrifuged at room temperature and 500 *g* for 5 min, after which the supernatant was removed and homogenized in 2 ml of buffer containing 1% SDS (w/v), 0.1 M Tris–HCl, pH 6.8, 20 mM dithiothreitol (DTT) and 1 mM phenylmethylsulfonyl fluoride. The homogenate was centrifuged at 15,000 *g* for 10 min (4 °C). The supernatant was subjected to phenol extraction as previously described [15]. The resulting phenol phase (containing proteins) was precipitated with 5 volumes of 0.1 M ammonium acetate in methanol for 1 h (−20 °C) and centrifuged at 20,000 *g* for 10 min (4 °C). The protein pellet was washed twice with cold acetone, air-dried, and resuspended in 2-DE rehydration buffer (7 M urea, 2 M thiourea, 4% (w/v) CHAPS, 20 mM DTT, and 1% (v/v) IPG buffer).