A tomato chloroplast-targeted DnaJ protein, SlDnaJ20 maintains the stability of photosystem I/II under chilling stress

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ABSTRACT

DnaJ proteins are key molecular chaperones that act as a part of the stress response to stabilize plant proteins, thereby maintaining protein homeostasis under stressful conditions. Herein we used transgenic plants to explore the role of the tomato (Solanum lycopersicum) SlDnaJ20 chloroplast DnaJ protein in to the resistance of these proteins to cold. When chilled, transgenic plants exhibited superior cold resistance, with reduced growth inhibition and cellular damage and increased fresh mass and chlorophyll content relative to control. These transgenic plants further exhibited increased Fv/Fm, P700 oxidation, \( \Phi_R^O \) and \( \Delta_R^O \) relative to control plants under chilling conditions. Under these same cold conditions, these transgenic plants also exhibited higher levels of core proteins in the photosystem I (PSI) and II (PSII) complexes (PsaA and PsaB; D1 and D2) relative to control wild-type plants. Together these results suggested that the overexpression of SlDnaJ20 is sufficient to maintain PSI and PSII complex stability and to alleviate associated photoinhibition of these complexes, thereby increasing transgenic plant resistance to cold stress.

1. Introduction

Cold stress is a common threat to a variety of terrestrial plants, with thermophilic vegetable crops including tomatoes (Solanum lycopersicum), cucumbers (Cucumis sativus), and bell peppers (Capsicum annuum) being particularly susceptible to chilling. Exposure to cold can impair plant growth and development, reducing both yield and quality. As photosynthesis is the key mechanism whereby plants accumulate biomass, this pathway is frequently targeted in an effort to improve crop yields. Importantly, owing to the complexity of the photosynthetic process it is very sensitive to cold stress.

There are two primary stages to photosynthesis – light and dark reactions. During the light reaction stage, solar energy is converted into an electric signal, with electrons moving through the electron transport chain in the thylakoid membrane of chloroplasts, thereby facilitating the movement of H\(^+\) ions into the thylakoid cavity and generating a proton gradient that can be harnessed to mediate ATP synthesis during the dark reaction. The photosystem I (PSI) and photosystem II (PSII) complexes are key components of the electron transport chain, with the former being a multi-protein pigment complex that catalyzes electron transfer from plastocyanin (PC) to ferredoxin (Fd) in a series of steps. PSII is additionally a multi-pigment protein complex which facilitates the transfer of electrons from water molecules to plastoquinone (PQ) in the thylakoid membrane. Cold temperatures have been shown to destroy chloroplasts and to thereby adversely impact PSI and PSII stability, disrupting photosynthesis, leading to PSI and PSII photoinhibition. In the presence of light, the PSI D1 protein undergoes rapid turnover such that following the end of PSI photoinhibition this protein can be rapidly restored to mediate the recovery of photosynthetic activity. However, the PSI complex lacks any similar high turnover proteins, and as such following the termination of PSI photoinhibition many proteins must be synthesized de novo resulting in delayed recovery of photosynthetic activity.

DnaJ proteins are key proteins that can act either on their own or in concert with Hsp70 proteins to mediate essential activities within cells such as protein folding, assembly, or degradation. Many studies have highlighted the importance of these DnaJ proteins for plant stress responses, with DnaJ proteins being expressed in nucleus, cytoplasm, endoplasmic reticulum (ER), mitochondria, and chloroplasts of cells. DnaJ proteins that localize to chloroplasts are well-characterized as being important mediators of plant stress responses. For example, the tobacco DnaJ protein Tsip1 is a zinc finger protein that is important for enhancing tobacco resistance to pathogenic or saline stress conditions. The AtJ8, AtJ11 and AtJ20 DnaJ proteins are all known to mediate photosynthetic pigment stability under high light conditions. The LeCDJ1 DnaJ protein is localized to chloroplasts and has been found to maintain PSII functionality under cold stress conditions. The SICDJ2 protein localizes to chloroplasts and in response to heat stress serves to preserve Rubisco activity. While in Chlamydomonas reinhardtii, ZnJ6 is a thylakoid-associated DnaJ-like chaperone that assists in contributing to stress endurance, redox maintenance and photosynthetic balance.

SlDnaJ20 is a DnaJ protein that localizes to chloroplasts in tomatoes, and that has been found to be upregulated in response to cold stress conditions. Herein we found that SlDnaJ20 overexpression was associated with enhanced PSI and PSII complex...
stability, thereby reducing the photoinhibition of these complexes and enhancing transgenic tomato resistance to cold stress.

2. Materials and methods

2.1. Plant growth under cold stress conditions

We assessed the growth of seedlings of either wild type (WT) tomatoes (S. lycopersicum cv. L-402) or those the T2 generation of three different transgenic lines overexpressing SlDnaJ20 (OE2, OE6, and OE9). These seeds were initially planted in Murashige and Skoog medium, and were placed in a GPJ-400 (Dongpeng Instruments, Jiangshu, China) light incubator with a 16:8 hour light/dark cycle at 25°C, with a ~100 μmol·m−2·s−1 photon flux density (PFD). After 10 days, half of these seedlings were transferred to an incubator that was identical except for the temperature, which was set to 4°C, while the remaining seedling served as controls. Plants were imaged 5 days later, and control plants were then added to a quartz sand medium and transitioned to a greenhouse (25°C, 16 h light/dark, 55%–65% relative humidity, 200 μmol·m−2·s−1 PFD) where they were twice weekly administered Hoagland’s nutrient solution. After a further 4 week growth period, WT and transgenic plants were incubated at 4°C with ~100 μmol·m−2·s−1 PFD for 48 h and were then imaged.

2.2. Cell viability and physiological measurements

Cells were stained using Trypan blue as in previous studies. Both growth inhibition and cold resistance index values were derived based upon previously detailed approaches, while work by Kong et al. was used to guide measurements of leaf chlorophyll content, relative electrical conductivity (REC), and malondialdehyde (MDA) levels.

2.3. Assessment of Chlorophyll a fluorescence

A Handy PEA (Hansatech Instruments, Norfolk, UK) was used to measure chlorophyll a fluorescence as in previous studies, with PSII Fv/Fm calculated based upon the following: (Fm−Fo)/Fm.

2.4. Assessment of chlorophyll fluorescence

A FMS-2 (Hansatech, Cambridge, UK) was used to measure chlorophyll fluorescence as in previous studies, with qI defined as Fm/Fmr-1, and qE defined as Fm/Fm′-Fm/Fmr.

2.5. Western blotting

Proteins were extracted from the thylakoid membrane as in previous studies, with Western blotting conducted based on protocols published by Wang et al. PsaA, PsaB, D1, D2, and Actin (against 100 amino acids of recombinant actin conserved peptide) antibodies were purchased from Agrisera Company (Umea, Sweden).

2.6. Statistical analysis

SigmaPlot 12.5 (Systat Software, San Jose, CA, USA) and SPSS 18.0 (Chicago, IL, USA) were utilized for statistical testing.

3. Results

3.1 Overexpressing SlDnaJ20 improves tomato cold stress resistance

We have previously shown that chilling tomato plants to 4°C leads to the upregulation of SlDnaJ20. As such, we sought to observe the impact of this protein on tomato plant growth under cold stress conditions by observing the relative growth characteristics of WT and SlDnaJ20-overexpressing 10-day-old or 6-week-old plants in response to cold stress conditions. We found that at normal temperatures the WT and transgenic plants both grew normally with no significant differences in plant phenotypes or physiology (Figure 1). When plants were chilled for 4 days to 4°C, however, WT seedling growth inhibition was markedly more pronounced than was inhibition of the transgenic seedling growth (Figure 1a). We further confirmed seedling cold tolerance index values and growth inhibition rates (Figure 1, b and c). Similarly, after a 48 h cold stress period mature transgenic plants grew more readily than did WT controls (Figure 1d). Chlorophyll contents and fresh weight values were also significantly higher for transgenic plants relative to WT controls (Figure 1, e and f). As such, these results clearly demonstrated that overexpressing SlDnaJ20 can significantly enhance tomato cold resistance.

3.2. Overexpression of SlDnaJ20 reduces cold-mediated damage to cell membranes

Membrane damage is the primary site of cold-induced damage, with plant cell membranes transitioning from a liquid crystal state to a gel-like state. These changes markedly impair both membrane permeability and associated cellular functionality. To assess cell membrane changes in transgenic plants, we used Trypan blue staining which revealed similar staining when cells were grown at normal temperatures, but reduced blue staining for SlDnaJ20-overexpressing plants under cold conditions relative to WT controls consistent with reduced membrane damage (Figure 2a). We additionally analyzed the REC and MDA contents in cells under these same conditions as these two markers offer insight into the degree of cellular damage. As with the trypan blue staining, at control temperatures WT and transgenic plants exhibited comparable levels of these two markers, whereas at 4°C the levels of both REC and MDA rose, with increases being significantly higher in WT plants relative to transgenic plants (Figure 2, b and c). These findings thus revealed that elevated levels of SlDnaJ20 can reduce cell membrane damage in response to cold stress.

3.3. Overexpressing SlDnaJ20 reduced PSI and PSII photoinhibition in response to cold stress

The extent of PSII photoinhibition is commonly measured based upon maximal photochemical efficiency (Fv/Fm). When grown at normal temperatures, Fv/Fm values did not
**Figure 1.** Cold resistance in 10-day-old and 6-week-old tomato plants. (a) 10-day-old seedlings were grown at either 25°C or 4°C for 5 d prior to imaging. (b) Cold resistance index. (c) Growth inhibition. (d) 6-week-old plants were grown at either 25°C or 4°C for 48 h. (e) Plant fresh weight. (f) Plant total chlorophyll content.

**Figure 2.** Measurement of cell damage in 6-week-old WT and transgenic plants. (a) Trypan blue staining, with plants incubated for 24 h at 25°C and 4°C shown in the upper and lower images, respectively. (b) REC. (c) MDA content.
differ significantly between WT and transgenic plants, whereas these values declined in all plants in response to cold stress, with decreases in WT plants being significantly larger than those in transgenic plants (Figure 3a). This was consistent with SlDnaJ20 mediating reduced PSI photoinhibition in response to cold stress. There are multiple potential mechanisms whereby this protein may have reduced Fv/Fm values in this context, including changes in qL and qE values. We found that as the duration of cold exposure increased qL values in WT plants to a greater extent than in transgenic plants (Figure 3b). Elevated qL values are consistent with more substantial photo-inhibition in WT plants under cold stress conditions relative to transgenic plants. In contrast, qE values did not differ significantly between these two plant groups, indicating that the elevated Fv/Fm values in transgenic plants may be a result of decreased qL values in these plants relative to WT controls (Figure 3c). We additionally analyzed three PSI activity-related fluorescent parameters (P700 oxidation, \( \varphi_{\text{Ro}} \) and \( \delta_{\text{Ro}} \)) in these plants and observed no differences in these values between WT and transgenic plants under normal growth conditions. In response to cold stress, however, transgenic plants exhibited less substantial reductions in P700 oxidation and \( \varphi_{\text{Ro}} \) relative to WT controls, with a correspondingly increased degree of \( \delta_{\text{Ro}} \) elevation in these transgenic lines (Figure 3d, e and f). This thus suggested that overexpressing SlDnaJ20 can reduce cold stress-induced PSI and PSII photoinhibition.

3.4. Overexpressing SlDnaJ20 reduces cold-induced PSI/PSII complex damage

Finally, we sought to explore the mechanistic basis for SlDnaJ20-mediated reductions in cold-induced PSI and PSII photoinhibition in tomato plants via analyzing levels of core proteins involved in these two complexes. Under normal temperatures, the core proteins in the PSI (PsaA and PsaB) and PSII (D1 and D2) complexes did not differ significantly between WT and transgenic plants (Figure 4). After a 24 h exposure to cold stress, however, transgenic plants exhibited significantly higher levels of PSI and PSII core complex proteins relative to WT controls, suggesting that overexpressing SlDnaJ20 helps to better maintain functionality of the PSI/PSII complex in response to chilling.

4. Discussion

Plant DnaJ proteins are widely expressed chaperone proteins that mediate environmental stress responses. While proteins in this family have been identified in a wide range of plants including rice, maize, and wheat, DnaJ expression and functionality in tomatoes are less well understood, particularly in the context of cold stress. We had previously found that incubation of tomato plants at 4°C led to induction of SlDnaJ20. As such, we explored the functional relevance of this DnaJ protein in tomato cold stress responses in both seedlings and mature plants, revealing that overexpressing SlDnaJ20 enhanced transgenic tomato cold resistance significantly (Figure 1).

Cold stress can adversely impact many stages of plant growth, including germination, photosynthesis, yield, and quality. Extreme or prolonged cold stress can lead to significant plant death. In this study, we found that transgenic tomato plants exhibited reduced levels of cellular damage in response to cold stress than did WT controls, suggesting that overexpressing SlDnaJ20 can protect tomato plants from cold-induced stress and associated damage (Figure 2). Cold conditions can impair photosynthesis, with inhibition of the PSI and PSII complexes being particularly pronounced upon chilling. We previously found SlDnaJ20 to localize to chloroplasts, consistent with our findings in the present study where in we observed significantly reductions in cold-induced decreases in PSII complex efficiency as measured based upon Fv/Fm in transgenic plants relative to WT controls (Figure 3a). Less substantial increases in qL values in transgenic
plants were also consistent with this phenotype (Figure 3b), indicating that overexpressing SlDnaJ20 reduced cold-induced PSII photoinhibition. With respect to PSI complex activity, we similarly found that transgenic plants exhibited less severe cold-induced decreases in P700 oxidation and \( \phi_{\text{Fv}} \) values relative to WT controls (Figure 3, d and e), while cold-induced increases in \( \delta_{\text{Fv}} \) were significantly larger than those in WT plants. These results thus revealed that overexpressing SlDnaJ20 can relieve cold-induced PSI and PSII photoinhibition, suggesting a role for this protein in cold stress responses.

Cold stress can mediate the destruction of key components of the PSI and PSII complexes, thereby inducing profound photoinhibition.\(^5\)\(^6\) Both of these complexes are vital components of the photosynthetic electron transport chain, and are highly susceptible to cold stress.\(^6\) In the present report we found that overexpressing SlDnaJ20 reduced cold-induced damage to both the PSI (PsaA and PsaB) and PSII (D1 and D2) complexes (Figure 4). We have previously shown SlDnaJ20 to interact with cpHSP70 in tomatoes.\(^19\) Together these findings thus suggest that SlDnaJ20 functions as a molecular chaperone to enhance protein folding, assembly, or stability under cold stress conditions.

In summary, overexpressing SlDnaJ20 can effectively reduce the cold-induced photoinhibition of the PSI and PSII complexes via increasing their stability, thereby enhancing transgenic tomato resistance to cold stress.

**Abbreviations**

Fd, ferredoxin; MDA, malondialdehyde; PC, plastocyanin; PFD, photon flux density; PSI, photosystem II; PSII, photosystem I; PQ, plastoquinone; REC, relative electrical conductivity; SlDnaJ20, *Solanum lycopersicum* DnaJ protein 20.

**Author contributions**

GD Wang and YS Zhu conceived and designed the study; GH Cai supervised the experiments; YJ Xu and SX Zhang conducted the experiments; TT Chen, G Liu and ZY Li analyzed the data, GH Cai drafted the original manuscript. All of the authors have read and approved the final manuscript.

**Disclosure statement**

No potential conflict of interest was reported by the author(s).

**Funding**

This work was supported by the Natural Science Foundation of Shandong (ZR2022QC240), the Natural Science Foundation of Rizhao (RZ2021ZR19), the Doctor Startup Foundation of Jining Medical University (600940001), the Research Fund for Lin He’s Academician Workstation of New Medicine and Clinical Translation in Jining Medical University (JYHL2019MS12).

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**Figure 4.** Western blot analysis of PSI and PSII core proteins. (a) PSI and PSII core protein levels in 6-week-old plants incubated for 48 h at 25°C and 4°C as assessed via Western blotting. (b) Quantitative image analysis of protein content in (a) using a Tanon Digital Gel Imaging Analysis System.
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