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OVEREXPRESSION OF MsDNAJ-PROTEIN ENHANCES DROUGHT TOLERANCE IN TRANSGENIC TOBACCO PLANTS

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ABSTRACT

As a co-chaperone DNAJ proteins play pivotal role in abiotic stress responses in plants, but the biological functions of DNAJ are poorly documented. In order to reveal the function of MsDNAJ proteins, physiological and molecular responses were investigated using transgenic tobacco plants. In this study, we showed that the transcript level of transgene MsDNAJ was elevated under drought treatment. The ectopic expression of MsDNAJ transgenic tobacco exhibited lower malondialdehyde (MDA) and hydrogen peroxide (H_2O_2) under drought condition. Moreover, the transgenic plants showed better growth with high chlorophyll fluoresce (Fv/Fm) and proline content compared to wild type (WT). In addition, overexpression of MsDNAJ improved drought stress tolerance in transgenic plants. These results suggest that overexpression of tobacco MsDNAJ protein gene enhanced drought tolerance in transgenic tobacco plants.

Keywords: MsDNAJ, Drought stress, Tolerance, Transgenic tobacco

INTRODUCTION

Drought stress greatly impairs on plant growth, development and yield (Jain and Chattopadhyay, 2013). The rate of photosynthesis is inhibited by drought stress, resulting cellular injury, membrane damage, subsequently changes proteins and enzymes in cells. In stress condition, plants produced several free radicals (e.g. ROS), which lead to induce oxidative damage of lipids, while a series of physiological and molecular alterations are occurred (Sharma *et al*., 2012). However, plants exhibit numerous enzymatic and non-enzymatic components for scavenging of cellular ROS. Low molecular weight osmolites including sugar, proline play pivotal role for the ROS homeostasis under drought stress (Jain and Chattopadhyay, 2013).

Drought stress can be avoided in plants by adopting several strategies including mass screening, marker-assisted selection, and exogenous application of osmoprotectants as well as molecular breeding approach. Transgenic engineering approaches in plants would be effective way to the development of new variety to improve drought tolerance. It has been reported that molecular chaperones like as heat-shock proteins (Hsps) can cope with stress-induced denaturation of other proteins. Several Hsps/chaperones families are recognized including the Hsp40 (DnaJ), Hsp60 (chaperonins), Hsp70 (DnaK), Hsp90, Hsp100 (Clp) and the small Hsp (sHsp) family (Wang *et al*., 2004). The DnaJ proteins are known as heat shock proteins 40 (Hsp 40s), which are considered as co-chaperones of heat shock proteins 70 (Hsp70s). DnaJ was previously characterized from *E. coli*, this protein is located in several compartments including chloroplast, mitochondria, cytosol, nucleus and plasma membrane (Ranjan and D'Silva, 2009). Research has been taken the Dnaj proteins AtDjB1 that contributes in thermotolerance through protecting cells from oxidative damage in *Arabidopsis* (Zhou *et al*., 2012).

DnaJ proteins are involved in numerous stress responses that play vital roles in growth and development (Bekh-Ochir *et al*., 2013). However, only few reports have been found on concerning to the role of DnaJ proteins for stress tolerance in plants. Tobacco is an important model crop for plant based molecular research. Tobacco is sensitive under drought stress that reduces plant growth, development and productivity. Therefore, it is imperative to emphasize study on physiological and molecular responses of tobacco plants to drought stress. We previously cloned and characterized DNAJ-proteins from alfalfa (MsDNAJ) plant and introduced in tobacco plants. In this study, the main objectives were to investigate physiological and molecular responses of MsDNAJ transgenic tobacco plants in terms of drought stress tolerance.

MATERIALS AND METHODS

Plant growth and treatment: Wild type (WT) and transgenic tobacco plants (*Nicotiana tabacum* L. cv. SR-1) were grown in horticulture potting mixed with 16/8 h (day/night) photoperiod, 200 $\mu mol^{-2}s^{-1}$ photon flux density (PFD) at 25°C in growth chamber. The plants were irrigated initially with Hoagland nutrient solutions. Four week-old WT and transgenic tobacco plants were exposed to drought stress for 12 days followed by 6 days

recovery to analysis. Control plants were irrigated normally. Simultaneously, one more group of plants was sampled at day 12 followed by 0, 6, 12 and 24 h for gene expression analysis. The plant leaves were harvested after completing drought treatment then quickly kept in liquid nitrogen for further analyses.

Measurement of chlorophyll florescence: Chlorophyll florescence was measured using portable fluorometer FMS2 (Hansatech, King's Lynn,UK). Tobacco plants were adapted at dark for 30 minutes prior to measurement. The formula [Fv/Fm=(Fm-F0)/Fm] was used for calculating chlorophyll florescence, where Fv, Fm and F0 are represent the relative, maximum and minimal florescence respectively.

Determination of malondialdehyde (MDA) content: Malondialdehyde (MDA; $\varepsilon = 155\ mM^{-1}\ cm^{-1}$) content was determined with the thiobarbituric acid-reactive substances (TBARS) following by the method of Rahman *et al.* (2014). Briefly, 0.5 g powder of leaves was mixed properly with 4 ml of 0.1% (w/v) trichloroacetic acid (TCA). The centrifugation was performed at 13000× g for 5 min, then 0.5% (w/v) thiobarbituric acid (TBA) was prepared by adding 20% TCA, and 1 ml supernatant was added to 4 ml TBA. The solution was kept at 95°C for 25 min for heating and then transferred on ice for 5min. Finally, the centrifugation was conducted for 15 min again, and 1 ml supernatant was taken from each sample. The absorbance was measured at 532 and 600 nm, respectively. The MDA level was calculated using a coefficient 155 $mM^1\ cm^1$.

Measurement of hydrogen peroxide (H_2O_2): In order to determine H_2O_2 level, 0.5 g powder of drought treated leaf sample was homogenized using a phosphate buffer (pH 6.8). The centrifugation, extraction of H_2O_2, determination of absorbance, and calculation H_2O_2 accumulation were performed following the protocol of Rahman *et al.* (2015).

Estimation of proline content: Proline was measured following by the method of Rahman *et al.* (2014). Briefly, 1.0 g tobacco leaves power was mixed properly with 10 ml of 3% sulfosalicylic acid, that filtered by centrifugation at 13000× g for 5 min. Subsequently, 2 ml filtrate; 2 ml acid ninhydrin (AN), 2 ml of glacial acetic (GAA), and 4 ml toluene was added in a 15 ml tube and centrifuged for 5 min. Subsequently 1 ml of solution was used to read the absorbance at 520 nm. The L-proline was considered as a standard for calculating of proline content in leaves.

Analysis of MsDNAJ-overexpressing tobacco by northern blot: Northern blot was performed as described previously by Lee *et al.* (2011). In this study, we investigated the temporal expression of MsDNAJ in tobacco leaves under drought stress. Total RNA was extracted from the tobacco leaves tissue using an extraction kit (Qiagen, USA). Finally, 10 µg of RNA sample was run on a 1.2% agarose gel migration set-up. Gene-specific probes were labeled with [α-^{32}P] dCTP following the method of Lee *et al.*(2018).

Statistical analysis: The data of the physiological indices were analyzed using software (SPSS v20.0, USA). The values were considered significant at the $P \leq 0.05$ level. All data were presented as mean ± SE of at least three independent experiments.

RESULTS

Effect of drought stress on chlorophyll florescence in tobacco leaves: The chlorophyll florescence (Fv/Fm) efficiency one of the key indicators for physiological studies in plants. As shown in Fig. 1a, the Fv/Fm was declined in response to drought stress. It reduced about 40% at day12 without water in WT plants while the little changes were found in transgenic plants. A wilting phonotype was highly observed for WT compared to transgenic plants. However, the transgenic plants were started to regrowth after 6 days recovery while only few WT plants were survived.

Effects of drought on lipid peroxidation in tobacco leaves: The malondialdehyde (MDA) level is a correspondence of lipid peroxidation is an indicator of ROS accumulation (Rahman *et al*, 2016). So, the MDA level was measured in tobacco leaf samples as a consequence of ROS (e.g. H_2O_2) level that induced by drought stress. As showed in Fig. 1b, the MDA content increased in tobacco leaves that prolonged with drought stress. The MDA content was approximately three fold higher in WT plants response to drought compared to normal condition. However, as a consequence of MDA, the H_2O_2 content was increased that about double in WT plants under drought compared to normal condition (Fig.1c).

Effect of drought on proline content in tobacco leaves: Proline accumulation is a key of physiological response during abiotic stress condition in plants. Proline contributes to maintain the stability of sub-cellular structure, scavenges free radicals, modulates cellular function and triggers gene expression. As showed in Fig.1d the proline content increased in WT tobacco plants in stress condition compared to normal condition. The value of proline accumulation was approximately 2.5 fold higher in WT stressed plants than control plants. However, the high proline content was observed in transgenic lines that about 1.3 fold higher compared to WT during drought stress, while no significant differences were observed among them in normal condition.

Morphological changes of tobacco plants under drought stress: To compare the phenotypic alterations between transgenic MsDNAJ lines and WT, we conducted drought tolerance bioassay. Phenotypically, the transgenic MsDNAJ tobacco lines showed mild wilting after 12 days of drought stress, whereas WT lines exhibited chlorosis and severe wilting (Fig. 2a). The drought recovery (6 days) performance was better in transgenic lines than WT (Fig. 2b).

Expressions of MsDNAJ-protein under drought stress: To further investigate the expression of MsDNAJ in response to drought stress, we performed Northern blot analysis. As showed in Fig. 3, the result exhibited the induction of MsDNAJ transcript in leaves under drought stress. In particular, the transcript of MsDNAJ was induced considerably at 6h of drought treatment while decreased at 12h followed by 24h. However, the MsDNAJ transcript exhibited a drought relating expression pattern that showed a disparity in tobacco plant leaves.

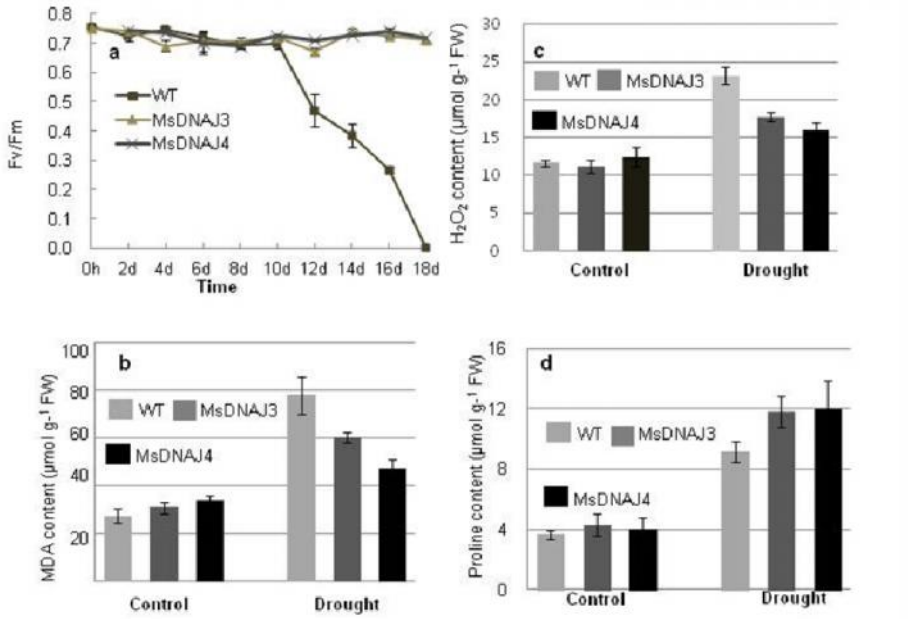

Fig.1. Physiological responses of tobacco plants under drought stress. Effects of drought stress on chlorophyll florescence (Fv/Fm) in leaves of wild type (WT) and MsDNAJ-transgenic lines (a). Effects of drought on MDA (b), H_2O_2 (c) and proline (d) content in tobacco leaves. Data represents the means and standard deviation (SD) of three independent experiments.

Fig.2. Phenotypes of tobacco plants in bioassay for drought stress tolerance. The morphological changes of WT and MsDNAJ transgenic tobacco lines were exhibited after 12 days of prolonged drought treatment (a). The photograph shows drought treated tobacco lines after 6 days recovery (b). Four-week-old WT and MsDNAJ-transgenic lines were exposed to drought stress as described in "Materials and Methods" section.

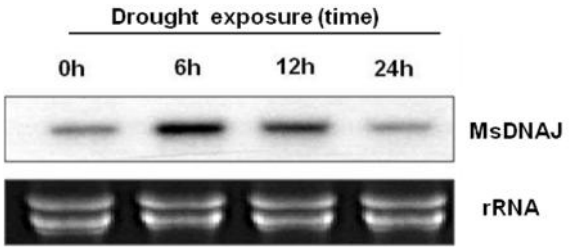

Fig.3. Northern blot analysis of MsDNAJ tobacco plants in response to drought stress. RNA was extracted from non-treated control (0), drought treated MsDNAJ lines with 6, 12 and 24 h intervel at 12 days of drought treatments. Total RNA (10µg) was separated by electrophoresis on an agarose gel, blotted on a nylon membrane and hybridized with ^{32}P-labeled gene specific cDNA probe.

DISCUSSION

Drought inhibited photosynthetic activity in tobacco: A plant response to drought is a complex phenomenon. It has been well documented that plant sub-cellular compartments including chloroplast, mitochondria are greatly affected by drought stress (Bigras, 2005). The photoinhibition data of WT tobacco leaves indicates that the integrity of subcellular compartments was greatly affected by drought stress. Generally, photosynthetic activities in tissues are inhibited by the imbalance of light absorption and utilization. In stress condition, often causes the delayed consumption of light resulting ROS production that injurious for plant tissues. Previous study suggests that LeCDJ2, a DNAJ protein induced the reduction in the photosynthetic capacity in tobacco plant under drought stress (Wang *et al*., 2014). However, in our study the induction of photosynthetic activity and the overexpression of MsDNAJ indicate the impact on photoinhibition that was alleviated in transgenic lines.

Lipid peroxidation and osmoregulation in response to drought stress in tobacco: The free radicals including ROS are mainly synthesized during stress condition in plants. The ROS attacks in plant cell whereas proteins, enzymes and other important macromolecules were greatly impaired (Sharma *et al*., 2012). The high content of MDA and H_2O_2 in WT plants indicate oxidative damage, cellular injury and lipid peroxidation occurred in plant cells. In opposition, drought stress comparatively alleviated higher in MsDNAJ transgenic lines. High accumulation of proline in transgenic line involved in regulation of osmotic stress indicates proline can regulate the osmotic stress under drought. Over all, these findings indicate that MsDNAJ transgenic lines suffer from less oxidative damages under drought condition which is correlate with increased proline, reduced MDA and H_2O_2 levels. Moreover, the overexpression of MsDNAJ alleviates drought tolerance in transgenic lines. The MsDNAJ is stress inducible protein, we are repotting for first time in transgenic tobacco plants. However, OsACA6 a cold stress tolerant transgene that reported previously in tobacco plants (Kamrul Huda *et al.,* 2014).

Overexpression of MsDNAJ enhanced drought tolerance: The data of this study revealed the great increase of drought tolerance in MsDNAJ transgenic tobacco lines than WT, that was due to the function of J-protein preventing photoinhibition and cellular damages, and improves the drought tolerance in plant cell (Rajan and D'Silva, 2009).We propose the MsDNAJ helps to maintain the cellular homeostasis by protecting of cellular active proteins and macromolecules from drought injury, which triggers physiological and molecular alterations, and raises the level of proline, subsequently enhanced drought tolerance in tobacco.

Conclusion: Drought stress adaptation in plants is a complex process. Tobacco plants were greatly affected by drought. The overexpression of MsDNAJ tobacco plants alleviated drought stress by reducing the excess accumulation of malondialdehyde (MDA) and hydrogen peroxide (H_2O_2) which can protect photoinhibition during stress. Overexpression of MsDNAJ protein possibly activated alone or associated with other cellular proteins played pivotal role in stress tolerance. Moreover, the osmoregulation process was involved for the adaptation of transgenic tobacco to drought stress. Further efforts are going to understand the regulatory mechanisms of MsDNAJ response to heavy metal stress.

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