The 6th International Conference on Biochemistry and Molecular Biology

Effect of drought stress on growth and photosynthetic pigments in transgenic kdml 105 rice overexpressing *OsCam1-1* gene

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Abstract

Drought stress is one of the essential environmental stresses that affects crops by changing in morphological, physiological, and biochemical direction tactics of plants. The OsCam1-1 is a rice calmodulin protein which transduces Ca^{2+} signals to their target proteins that are important to regulate cellular and physiological responses. In this study, the 21 day-old rice seedlings were exposed to 20% (w/v) polyethylene glycol-4000 to mimic drought stress for 24 h and investigated growth and photosynthetic pigments in the transgenic rice overexpressing *OsCam1-1* gene under drought stress compared to the wild type (WT) and control transgenic KDML 105 transformed with blank vector (VC). The relative growth rate of roots and shoots, relative water content, chlorophyll *a*, and carotenoid contents were slightly decreased, while DPPH scavenging activity was significantly increased in transgenic rice under drought stress compared to WT and VC. In addition, transgenic rice showed less reduction in chlorophyll *b* and total chlorophyll than WT and VC under drought stress. Thus, the *OsCam1-1* gene overexpressing transgenic rice could protect growth and photosynthetic pigments under drought stress condition.

Introduction

In recent years, the global warming leads to the environmental stresses such as drought, heat, salinity, or cold stress which affect the yield and the living of plant. Drought is a damaging stress for crops by resulting in the altering of morphological, physiological and biochemical adaptation for the survival of plant.[1,2,3] Reactive oxygen species (ROS) such as superoxide anion ($O^{2\bullet-}$), hydrogen peroxide (H_2O_2), singlet oxygen ($^1O_2$), hydroperoxyl radical ($HO^{2\bullet-}$), hydroxyl radical ($OH^{\bullet}$), alkylperoxyl radical (ROOH), and alkoxyl radical ($ROO^{\bullet}$) are reactive molecules and toxicity to the cellular including protein damage, lipid membrane oxidation, and DNA detriment which bring about to cell death. ROS molecules are scavenged by antioxidant enzymes and non-antioxidant enzymes which relate to the antioxidant defense mechanism and the differentiation of ROS level rely on the environmental stresses.[4]

Rice is a main human food plant and widely used as a model of monocotyledons for researching about molecular biology. Khao Dawk Mali (KDML) 105 (*Oryza sativa* L.) has been recognized as Thai Hom Mali Rice in the world food trade, it has a fragrant odor and popularly plants in Thailand.[5] Thus, the demand of KDML 105 enhances every year in the world cuisine market.[6,7] Nevertheless, the rice production is seriously reduced in the result of the global warming effects that disturb adaptation strategies as well as cellular and developmental processes of leading to decrease fertility and grain quality.[8,9]

Calcium ion (Ca^{2+}) is a secondary messenger in the calcium signal pathway, plays as an essential role for plant development and plant stress tolerance.[10] Plant cells accumulate high

Ca^{2+} concentration in their cytosolic for responding to stress resistance. The Ca^{2+} sensors transduce the Ca^{2+} signals to their objective proteins leading to the protein conformation alterations which involve in their functions. The transgenic rice overexpressing *OsCam1-1* was constructed and showed that it was more resistant to salinity than the control plant.[11] However, the role of *OsCaM1-1* has not been reported in the condition of drought, heat, or cold stress. Here, the relative growth rate, the relative water content, the DPPH scavenging activity, and the photosynthetic pigments content were determined in response to drought stress in the transgenic rice overexpressing *OsCaM1-1* gene compared to WT and VC plants.

Methodology

Plant materials and stress treatments

WT seeds of KDML 105 rice cultivar were kindly given by Department of Agriculture, Ministry of Agriculture and Cooperatives (Bangkok, Thailand). Rice seeds of three transgenic KDML 105 lines overexpressing *OsCam1-1* (L1, L2, and L3) and VC were received from the research group 'Special Task Force for Activating Research (STAR) on Biochemical and Molecular Mechanism of Rice in Changing Environments' assisted by the Ratchadaphiseksompot Endowment Fund, Chulalongkorn University. Firstly, seeds were drenched in 6 % (v/v) sodium hypochlorite for 5 min and washed thoroughly with distilled water (DW). Then, they were germinated in DW in darkness for 5 d. Next, seeds were moved into a plant growth chamber (Human Lab, South Korea) under managed situations (temperature of 25 ± 2 °C, 16 h light (200 µmol m-2 s-1) / 8 h dark photo period, and relative humidity of 70 %) for 7 d. After that proliferating seeds were matured in Limpinuntana's nutrient solution[12] for 14 d. For drought treatment, the 21 d old seedlings were exposed to 20 % (w/v) Polyethylene glycol (PEG)-4000.[13,14] The seedlings were harvested after exposure to PEG for 24 h by roots and shoots were separately collected. Finally, the leaves were collected and maintained at -80 °C freezer (New Brunswick Scientific, England).

Relative growth rate determination

The sample seedlings were randomly selected and dried in an electric oven (Memmert, Germany) at 60 °C for 3 d. Then, the dry weight was recorded by an CP224S analytical balance (Sartorius, Germany). The relative growth rate (RGR) was calculated using the equation[15] : $RGR = (\ln W_2 - \ln W_1)/(t_2 - t_1)$; W_1 is dry weight at time t_1 and W_2 is dry weight at time t_2.

Relative water content determination

Leave samples were cut into small pieces 0.5 cm^2 and fresh weight (FW) was measured. Then, samples were soaked in DW for 24 h, after that turgid weight (TW) was read. Latterly, samples were dried in the electric oven at 60 °C for 72 h and the dry weight (DW) was recorded.[16] The reltive water content (RWC) was calculated following this formula: $RWC = (FW - DW)/(TW - DW) \times 100$

Measurement of DPPH scavenging activity

The DPPH scavenging rate was determined as described method of Szabo and coworkers[17] with some modification. Leave samples 0.05 g were crushed in liquid nitrogen by a Mixer Mill MM 400 (Retsch, Germany) and 1 ml of 95 % methanol was added into the pound sample tube. Then, the solutions were shaken at 3,000 ×g, 10 °C for 5 min using a Vibro shaker (Labinco BV, Netherlands) and centrifuged at 13,000 ×g, 25 °C for 5 min by a 5804R centrifuge (Eppendorf, Germany). The DPPH reagent 190 µl prepared from 10 ml of 0.6 mM DPPH and 45 ml of 95% methnol, was mixed with the sample solution 10 µl in 96 well plate and incubated in the dark for 30 min. The absorbance was read at 515 nm by using a Synergy H1 microplate reader (Biotek, USA). The DPPH scavenging rate was calculated following the equation: DPPH radical scavenging activity (%) = $(A_{control} - A_{sample}) \times 100/(A_{control})$

Measurement of Photosynthetic pigments contents

Leave samples 0.05 g were powdered in liquid nitrogen by a Mixer Mill MM 400 and homogenized with dimethylformamide at 5,000 ×g, 4 °C for 10 min. The absorbance of the supernatant was measured at 461, 647, and 664 nm.[18] The photosynthetic pigments were calculated following the equation:

chlorophyll *a* (chl *a*) content (µg/ml) = $[12.7(A_{664})] – [2.79(A_{647})]$

chlorophyll *b* (chl *b*) content (µg/ml) = $[20.70(A_{647})] – [4.62(A_{664})]$

total chlorophyll (total chl) content (µg/ml) = $[17.90(A_{647})] + [8.08(A_{664})]$

carotenoid content (µg/ml) = $4 \times [(A_{461}) + (0.46 \times A_{664})]$

Statistical analysis

Statistical analysis was operated by testing with a one-way analysis of variance (ANOVA) ensuing in the Duncan's new multiple range test ($P < 0.05$) using SPSS software version 16.0.

Results and Discussion

Effect of drought stress on RGR

RGR is the most significant parameter to indicate the plant growth.[19] In this study, the percentage of RGR of root and shoot was affected by drought stress (Figure 1a, b). They were slightly reduced in the transgenic rice overexpressing *OsCaM1-1*, while in the WT and VC, they were significantly reduced. This corresponds to the report of Jung and coworkers, the *O. sativa* calmodulin-like protein gene (*OsCML16*) enhances the root growth in transgenic rice under drought stress.[20] Similar to *OsCam1-1* which is a functional calcium-binding protein gene that displays as an important inheritance in plant calcium signals[21], *OsCML16* is a calmodulin-like protein (CML) which is an important gene in calcium signaling during abiotic stress.[20] And the report of Yin and coworker, the overexpression of *O. sativa* drought stress response-1 gene (*OsDSR-1*) shows the higher plant growth and higher survival under drought treatment in transgenic rice than the control plants. [22] Like *OsCam1-1* which has been reported that it acts as a salt stress sensor in salinity stress in rice[11], *OsDSR*-1 a CML genes which regulates growth and development of plants as well as responses to abiotic stresses such as salinity and drought.[22] Thereby, the overexpression of *OsCam1-1* encouraged in the reservation of RGR in transgenic rice under drought stress. Moreover, the transgenic rice overexpressing *OsCaM1-1* were found to grow and improve the plant adaptation to drought stress than WT and VC (data not shown).

Effect of drought stress on RWC

The RWC, known as the relative turgidity, is an indicator of the water status of leaves.[23] As showed in Figure 1c, drought stress affected on RWC level of all plants. However, the three transgenic plants showed insignificant reduction in RWC level when compared to WT and VC. The result is consistent with the report of Wei and coworkers, the overexpression of rice calcium-dependent protein kiness 9 gene (*OsCPK9*) decreases in the RWC reduction under drought stress conditions in transgenic rice.[24] *OsCPK9* is a positive regulator of abiotic stress resistance and ABA sensitivity[24] that like to *OsCaM1-1* which plays a key role in ABA biosynthesis.[11] As the report of Munir and coworkers, the overexpression of *Solanum habrochaites* cold responsive calmodulin-like gene (*ShCML 44*) alleviates in the RWC reduction in transgenic tomato under drought stress.[25] *ShCML 44* is an important calcium sensors and showed greater resistance to abiotic stresses[25] that worked like *OsCaM1-1* which plays in various roles as signal transducers in controling various developmental processes to abiotic stresss in plants.[13]The results suggested that the overexpression of *OsCam1-1* helped to maintain the hydration state of leaves under drought stress.

Effect of drought stress on DPPH scavenging rate

ROS is the by-product which was formed from the physiological metabolisms, and was controlled by enzymatic and non-enzymatic antioxidant systems.[26] Drought stress increased an oxidative damage and the H_2O_2 accumulation that was reduced by DPPH-radical scavenging system.[27] In our study, DPPH scavenging rate was increased in all type plants by drought treatment (Figure 1d). However, DPPH scavenging rate was significantly increased in the three transgenic plants compared with WT and VC. This is consistent with the overexpression of *ShCML44* in transgenic tomato[25] and the overexpression of *OsDSR-1* in transgenic rice[22] that response to drought stress by up-regulating in the antioxidant enzyme activity. These results suggested that the overexpression of *OsCam1-1* enhanced the induction in DPPH scavenging rate under drought stress.

Effect of drought stress on photosynthetic pigment contents

Pigments such as chlorophylls and carotenoid contents are essentially related to the physiological function of leaves. For example, chlorophylls absorb and transfer the light energy, which can also be transfer by carotenoids, into the photosynthetic system.[28] In this study, the effect of drought stress on the photosynthetic pigment contents (chlorophyll *a*, *b*, total chlorophyll and carotenoid) were observed as shown in Figure 1e- h. All photosynthetic pigment contents significantly decreased in WT and VC under drought stress. But the reduction of all photosynthetic pigment contents in the transgenic rice under drought stress decreased slightly. These result is consistent with previous report by Li and coworkers, the overexpression of *Populus euphratica* calcinurin B-like gene (*PeCBL*) shows the better maintenance in the height growth rate and the chlorophyll content under drought stress in transgenic triploid white poplar.[29] *PeCBL* is a calcium sensor that plays an important role in decoding calcium transients[29] that is similar to *OsCaM1-1* which is a functional calcium-binding protein that exhibited essential relays in plant calcium signals.[21] In the same way, the overexpression of *OsCPK9*, a calcium sensor likes *OsCaM1-1*, exhibits a positive drought tolerance had been reported in transgenic transgenic rice by high keeping in the chlorophyll content and the elongation of plant shoot and root.[24] Thereby, the *OsCam1-1* gene is a positive regulator to the plant growth rate and the photosynthetic pigment content.

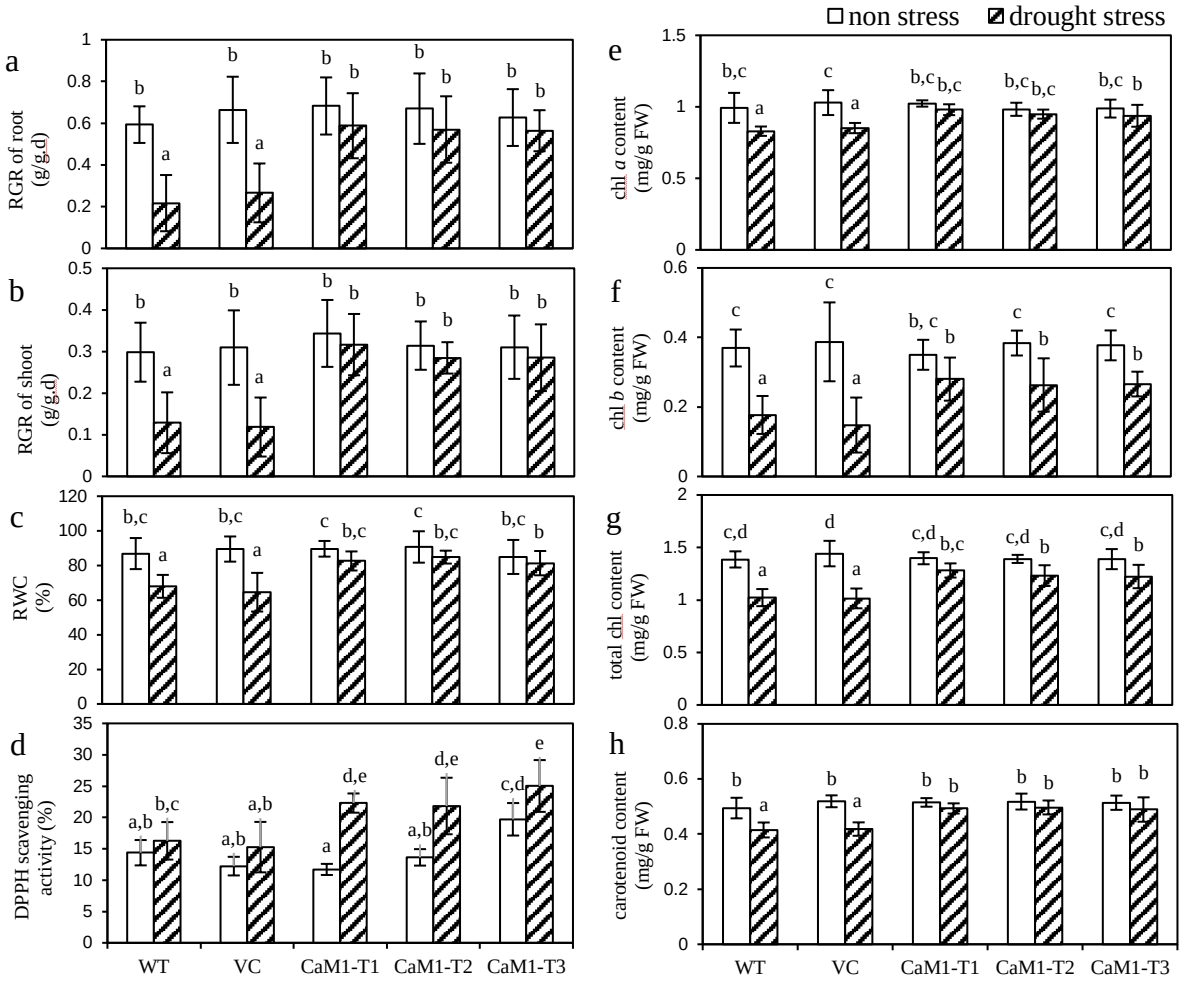

Figure 1. Effect of drought stress for 24 h. on RGR of root (a), RGR of shoot (b), RWC (c), DPPH scavenging activity (d), chl *a* (e), chl *b* (f), total chl (g), and carotenoid contents (h) in leaves of the three transgenic rice overexpressing *OsCaM1-1* gene (CaM1-T1, CaM1-T2, and CaM1-T3) when compared to wild type (WT), vector control (VC). Error bars represented standard deviation (n = 5). The different letters indicate significant differences at $p < 0.05$ according to Duncan's test.

Conclusion

The overexpression of *OsCam1-1* gene enhances drought tolerance in transgenic rice by up-regulating in the ability of DPPH scavenging activity and improving in increase of the water retention ability, accordingly maintaining in the photosynthetic pigment accumulation and plant growth rate. These results suggested that the overexpression of *OsCam1-1* is a positive indicator to drought resistance in rice.

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Acknowledgements

This research was supported by the 90th anniversary of Chulalongkorn University Fund (Ratchadaphiseksomphot Endowment Fund), the scholarship from Chulalongkorn University to develop research potential for the Department of Biochemistry, Faculty of Science, Chulalongkorn University (Ratchadaphiseksomphot Endowment Fund), and the scholarship from The King Prajadhipok and Queen Rambhai Barni Memorial Foundation.