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Author(s)	J. He and L. Qin

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LED quality impacts on plant growth and photosynthetic light use efficiency of halophyte vegetable *Mesembryanthemum crystallinum* grown under two saline conditions

J. He[1] and L. Qin

National Institute of Education, Nanyang Technological University, 1 Nanyang Walk, Singapore 637616

Abstract

There is very little study on the effects of LED quality on *Mesembryanthemum crystallinum* grown under different salinities. In this study, *M. crystallinum* was grown under red/blue (R/B) LED ratios of 0.9, 1.6. 2.0 and 2.8 with 100 and 500 mM NaCl and exposed to the same level of photosynthetic photon flux density, PPFD (290 µmol m^{-2} s^{-1}, 12 h photoperiod). Fourteen days after transplanting, plants with 100 mM NaCl had significantly higher values of growth parameters including shoot and root fresh weight, total leaf area and specific leaf area than with 500 mM NaCl. Grown with 100 mM NaCl under R/B 0.9, *M. crystallinum* had significantly lower shoot FW and total leaf area than under other R/B ratios. However, LED quality did not seem to affect the growth parameters of *M. crystallinum* grown with 500 mM NaCl. All plants had similar higher leaf succulence and water content but lower leaf dry matter content with 100 mM NaCl than with 500 mM NaCl. Crassulacean acid metabolism (CAM) acidity of plants grown with 500 mM NaCl was about 4-fold higher than with 100 mM NaCl, indicating induction and stimulation of CAM photosynthesis. Chlorophyll fluorescence F_v/F_m ratios were greater than 0.8 for all plants, suggesting that maximum efficiency of PSII was unaffected by LED quality and salinity. *M. crystallinum* grown with 500 mM NaCl had lower electron transport rate, ETR but higher non-photochemical quenching, NPQ than with 100 mM NaCl, implying that photosynthetic light use efficiency was affected when switching C_3 to CAM photosynthesis under high salt stress. *M. crystallinum* grown with100 mM NaCl had higher photochemical quenching, qP and ETR under R/B 0.9 than under R/B 2.8 and all plants with 500 mM NaCl. In conclusion, LED quality had different impacts on photosynthetic light-use efficiency of *M. crystallinum* grown under different salinities and thus resulting in different growth and productivity.

Keywords: CAM acidity, LED quality, *Mesembryanthemum crystallinum,* Photosynthesis; Salinity

INTRODUCTION

Due to the lack of available land, Singapore needs to develop vertical farming systems that can increase productivity of vegetables per unit land area. The facultative halophyte vegetable, *M. crystallinum* (ice plant) has been successfully grown in Singapore with vertical farming systems under different LED-lightings using freshwater by our research team. We have found that the *M. crystallinum* plants performed C_3 photosynthesis with adequate water supply. They had the higher shoot and root productivities with a faster leaf development under different combined red and blue LEDs compared to plants grown under red-LED alone (He, 2017; He et al., 2017).

[1]Corresponding author, email: jie.he@nie.edu.sg

Recently, we studied the impacts of different NaCl concentrations on *M. crystallinum.* It was found that *M. crystallinum* plants grown under 100 mM NaCl had the highest shoot fresh weight and largest total leaf area followed by plants at 0, 250 and 500 mM NaCl after transplanting for 20 days. To maximize its productivity, *M. crystallinum* requires certain level of salt such as 100 mM NaCl. The switch of C_3 to crassulacean acid metabolism (CAM) photosynthesis induced by high salinity was the main reason resulted in low productivity of *M. crystallinum* (He and Qin, 2020).

Salinity is the main factor that induces CAM photosynthesis in *M. crystallinum.* High irradiance as well as photoperiod and aging have also been reported to induce the switch of C_3 to CAM photosynthesis in *M. crystallinum* (Winter and Gademann, 1981; Lüttge, 2002; Hurst et al., 2004; Broetto et al., 2007). Broetto et al. (2007) reported that both salinity and high light intensity included CAM in *M. crystallinum.* They found that low light (200 μmol m^{-2} s^{-1}) with salt (400 mM NaCl) and high light (1000 μmol m^{-2} s^{-1}) without salt induced weak CAM. Potential quantum efficiency of photosystem II, PSII, F_v/F_m was not affected by any of these conditions, remaining at ⩾0.8, indicating that there was no acute photoinhibition. However, high light with salt resulted in stronger CAM with clearly reduced effective quantum yield of PSII, $\Delta F/F'_m$. and also caused photoinhibition reflected by reduced F_v/F_m ratio. Non-photochemical quenching, NPQ, remained <0.5 during the daily course for plants grown under low and high lights without salt and low light with salt but increased to 1–2 under high light with salt. Maximum electron transport rates, ETR_{max}, declined in plants under low light and high light with salt during the daily courses. These results indicate that photosynthetic capacity is reduced by the synergistic effects of salinity and high light.

Apart from light intensity, light quality is the most important variable affecting the light use efficiency for plant growth (He et al. 2015; Choong et al., 2018 He et al., 2018). However, there is very little study on the effects of light quality on *M. crystallinum* grown under different saline conditions. We hypothesized that responses of *M. crystallinum* to LED quality could be different under different salinities. Thus, in this study, *M. crystallinum* was grown under different LED spectra with 100 and 500 mM NaCl concentrations. It aimed to investigate the impacts of LED quality on shoot and root production, leaf growth and water status of *M. crystallinum* grown under two different salinities. Photosynthetic light use efficiency of *M. crystallinum* was also investigated. The findings would help advance existing understanding of ice plant physiology under different salinities and LED qualities. It could also help the growers to enhance productivity of ice plants at low production cost through the optimal selections of LED lighting and salinity.

MATERIALS AND METHODS

Plant materials and experimental design

After germination, *M. crystallinum* seedlings were placed under a photosynthetic photon flux density (PPFD) of 100 μmol m^{-2} s^{-1} (12 h photoperiod) provided by high-pressure sodium lamps for 4 weeks. Seedlings were then transplanted to indoor hydroponic systems. They were grown under different red/blue (R/B) LED ratios of 0.9, 1.6. 2.0 and 2.8 (defined as R/B 0.9, R/B 1.6, R/B 2.0 and R/B 2.8, Figure 1) and exposed to the same level of PPFD of 290 μmol m^{-2} s^{-1}, 12 h photoperiod. Under each of the LED spectrum, plants were grown under two different salinities by adding 100 and 500 mM NaCl, respectively to a full strength Netherlands Standard Composition (Douglas, 1979) with 2.2 ± 0.2 mS cm^{-1} conductivity and pH 6.0 ± 0.2. The room temperature and relative humidity were as 25℃/28℃ and 55%/60% (day/night), respectively.

Measurements of growth parameters and leaf water status

Fourteen days after transplanting (DAT), plants were harvested and the polyurethane cube was removed from the roots. The total leaf number was first recorded before leaf, shoot and root fresh weights (FW) were weighed separately using a weighing balane (Sartorius, Fisher General Specific Private Limited, Singapore). Total leaf area was also measured using the Area Measurement System (Delta T-Devices Ltd., England). All tissues were then wrapped individually in pre-weighed aluminium foil, dried at 80˚C for 5 days before measuring their dry weight (DW). Leaf succulence = L_{FW}/L_A with L_{FW} = leaf FW (g) and L_A, leaf area (cm^2). Leaf dry matter content (LDMC) and leaf water content (LWC) were estimated as LDMC = L_{DW}/L_{FW} and LWC = $(L_{FW} - L_{DW})/L_{FW}$ x 100%.

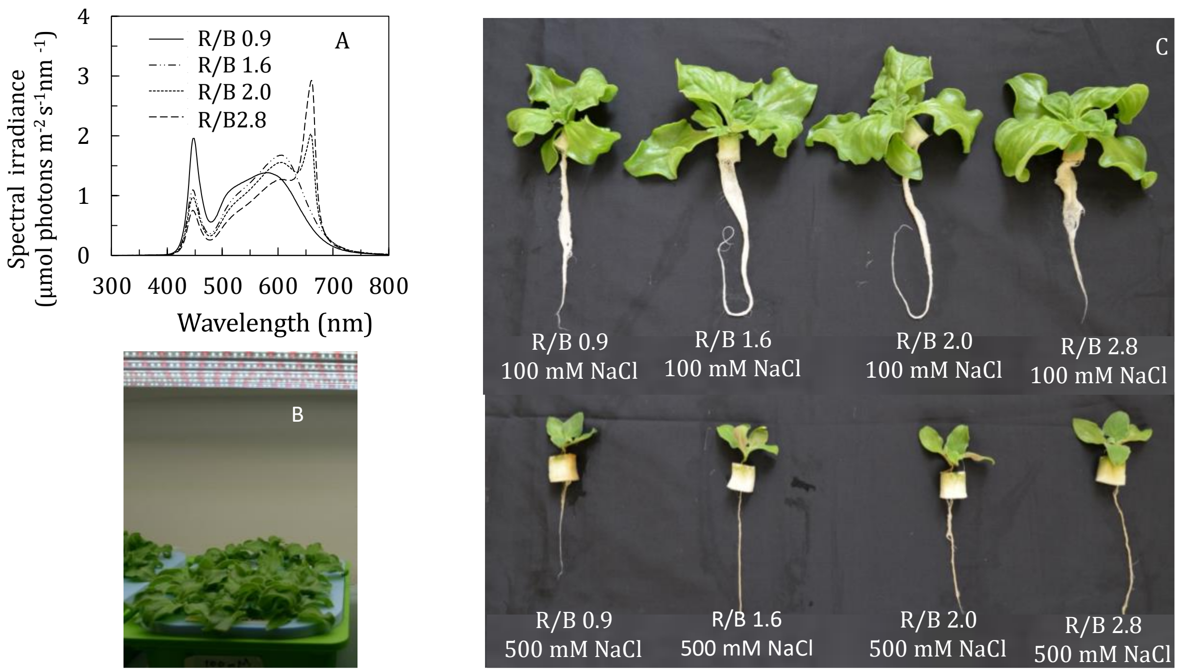

Figure 1 Light spectrum of LED with red/blue ratios of 0.9, 1.6 , 2.0 and 2.8 (R/B 0.9, 1.6. 2.0 and 2.8) were recorded at every 0.5 nm interval with a spectroradiometer (PS300, Apogee Instruments, USA) (A)*M. crystallinum* grown under R/B 2.8 with 100 mM NaCl for 14 days (B). *M. crystallinum* grown under different R/B LED ratios and NaCl concentrations for 14 days (C).

Measurement of chlorophyll fluorescence F_v/F_m ratio

The maximum efficiency of PS II was estimated by F_v/F_m ratio 14 DAT. All leaves were kept in darkness for15 min before the measurements during mid-photoperiod using the Plant Efficiency Analyser (Hansatech Instruments, UK) according to He et al. (2011).

Measurements of electron transport rate (ETR), photochemical quenching (qP) and non-photochemical quenching (NPQ)

The youngest fully expanded leaves were harvested 14 DAT and pre-darkened for 15 min before the measurements. ETR, qP and NPQ were measured using the Imaging-PAM Chlorophyll Fluorometer (Walz, Effeltrich, Germany) at 25°C in the laboratory. All images of fluorescence emission were digitized within the camera and via a Firewire interface (400 megabits/s) (Firewire-1394, Austin TX, USA) to a personal computer for storage and analysis. Measurements and calculations of ETR, qP and NPQ were described by He et al. (2011).

Determination of CAM acidity

Leaf discs cut from the youngest fully expanded leaves 14 DAT were placed into test tubes containing distilled water before immersing into a boiling water-bath for 15 min. The extract was subsequently titrated against 0.005 M NaOH(aq), using a few drops of phenolphthalein as an indicator. The volume of NaOH(aq) required to reach the end-point of titration was recorded. The leaf discs was then wrapped in an aluminum foil and dried in an oven at 80°C for 3 days before recording the DW. Titratable acidity was calculated using the formula: (0.005 × volume of NaOH(aq))/DW. The fluctuation of CAM acidity was calculated by obtaining the difference between titratable acidity immediately before and after the photoperiod (He et al., 2017).

Statistical analysis

One-way ANOVA was used to test for significant differences of different variances crossed with the four different LED spectra and two salinities. LSD multiple comparison tests were used to discriminate the means (IBM SPSS statistics 25, Release August 2017).

RESULTS AND DISCUSSION

Productivity and leaf growth

M. crystallinum grown with 100 mM NaCl concentration for 14 days had much higher shoot FW (Figure 2A) and root FW (Figure 2B) compared to those grown with 500 mM NaCl concentration. Regardless of LED spectral quality, all plants had similar shoot and root FW with 500 mM NaCl. However, grown under R/B ratio of 0.9, shoot FW of *M. crystallinum* was significantly lower than those of plants grown under R/B 1.6, 2.0 and 2.8 (Figure 2A). *M. crystallinum* grown with 100 mM NaCl had the significant higher root FW under R/B 1.6 than other R/B LED ratios. There were no significant differences in shoot/root ratio among all plants except for plants growth with 500 mM NaCl under R/B 2.0 (Figure 2C).

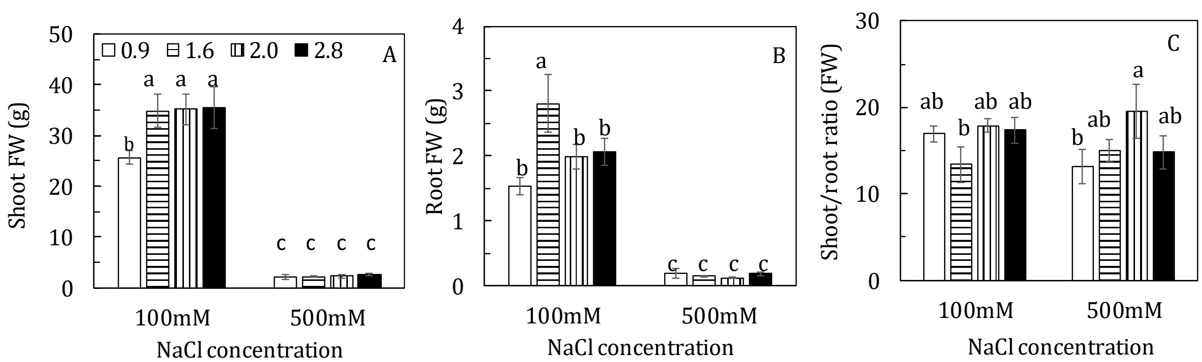

Figure 2 Shoot FW (A), root FW (B) , shoot/root ratio FW (C) of *M. crystallinum* grown under different R/B LED ratios with 100 and 500 NaCl mM for 14 days. Vertical bars represent the standard errors. Means with different letters are statistically different ($p < 0.05$; n=4) as determined by LSD multiple comparison test.

Grown with 100 mM NaCl, *M. crystallinum* had significant lower total leaf number (Figure 3A) under R/B 0.9 than under R/B 2.0 and 2.8**.** Total leaf area was significantly lower with 100 mM NaCl under R/B 0.9 than other R/B LED ratios (Figure 3B). For SLA, grown with 100 mM NaCl under R/B 0.9, the value was significantly lower than those under R/B1.6 and 2.0. However, all plants had similar values of leaf growth parameters with 500 mM NaCl regardless of LED quality (Figure 3). We have previously reported that *M. crystallinum* grew well under both fresh water (He et al., 2017) and saline conditions (He and Qin 2020). Halophytes generally require some salt for optimal growth (Flowers et al., 2008; 2010). In this study, it was also found that grown with 100 mM NaCl, *M. crystallinum* had significantly lower values of shoot FW and total leaf area under R/B 0.9 than under R/B 1.6, 2.0 and 2.8. However, LED quality had no significant impact on shoot and root productivity (Figure 2) and leaf growth (Figure 3) when grown under 500 mM NaCl. These results indicate that adverse effects of high salinity on plant growth attenuated the impacts of LED quality on *M. crystallinum*.

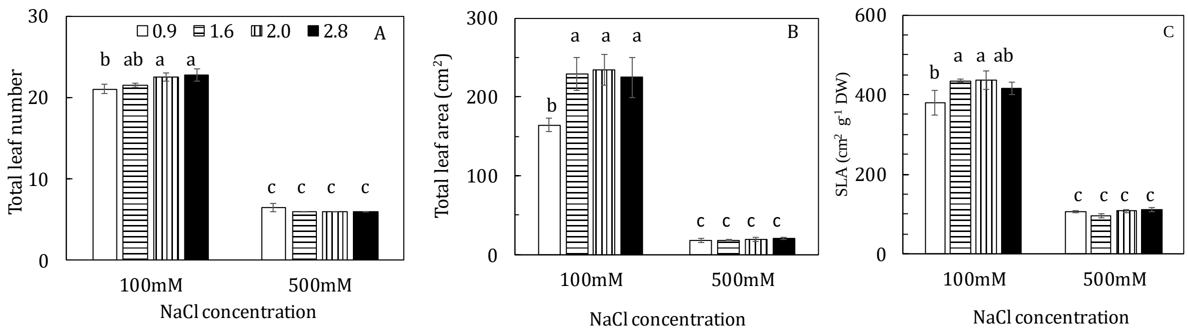

Figure 3 Total leaf number (A), total leaf area (B) and SLA (C) of *M. crystallinum* grown under different R/B LED ratios with 100 and 500 NaCl concentrations for 14 days. Vertical bars represent the standard errors. Means with different letters are statistically different ($p < 0.05$; n=4) as determined by LSD multiple comparison test.

Leaf water status

In this study, LED quality did not affect the leaf succulence of *M. crystallinum* grown with 100 mM or 500 mM NaCl. However, leaf succulence which was measured as the maximum water content expressed as fresh mass per unit of leaf area (g FW cm^{-2}) (Debez et al., 2004), was significant higher in *M. crystallinum* grown with 100 mM NaCl than with 500 mM NaCl (Figure 4A). Belkheiri and Mulas (2013) reported that increased salinity enhanced leaf succulence. The

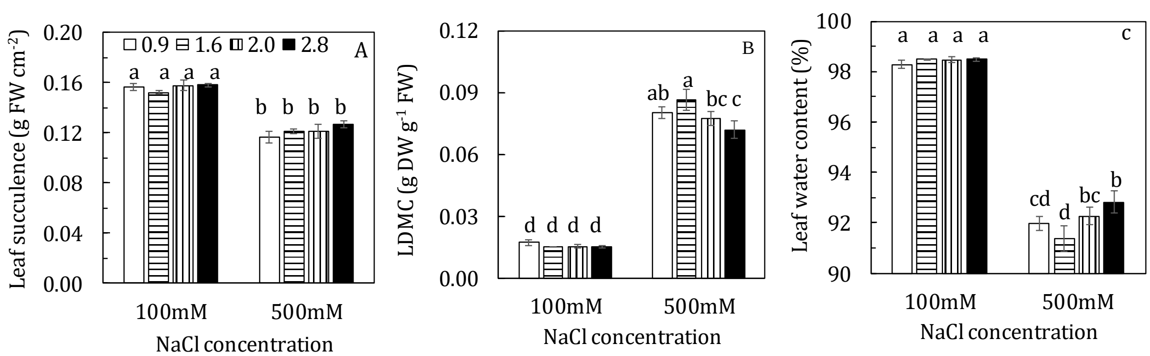

Figure 4 Leaf succulence (A), LDMC (B) , leaf water content (C) of *M. crystallinum* grown under different R/B LED ratios with 100 and 500 NaCl concentrations for 14 days. Vertical bars represent the standard errors. Means with different letters are statistically different ($p < 0.05$; n=4) as determined by LSD multiple comparison test.

concentration at which leaf succulence is high would be the optimal NaCl concentration for growth. A further increase in salinity to 500 mM NaCl used for this study resulted in decrease in growth and leaf succulence (Khan et al., 2000). *M. crystallinum* grown with 100 mM NaCl had higher SLA (Figure 3C) could be due to its lower LDMC (Figure 4B) but high leaf water content (Figure 4C).

CAM acidity and photosynthetic light use efficiency

CAM acidity of *M. crystallinum* grown with 500 mM NaCl was significantly higher than with 100 mM NaCl, indicating the switch of photosynthesis from C_3 to CAM upon high salt stress (Matsuoka et al., 2018). Grown with 500 mM NaCl under R/B 1.6, *M. crystallinum* had higher CAM acidity compared to that of R/B 2.0. There were no significant differences in CAM acidities among different LED R/B ratios with 100 mM NaCl (Figure 5A). All plants had F_v/F_m ratios greater than 0.8 (Figure 5B), implying that the maximum potential quantum efficiency of PS II was not affected by either LED quality or salinity in any plants. When CAM was induced in *M. crystallinum* by salt stress, Broetto et al. (2007) found that F_v/F_m ratio decreased. After subjecting *M. crystallinum* to salt stress for 14 days, however, Barker et al. (2004) did not observe any changes in F_v/F_m ratios. Our results further confirmed that there was no close relationship between photoinhibition measured by F_v/F_m ratio and the induction of CAM photosynthesis (Matsuoka et al., 2018).

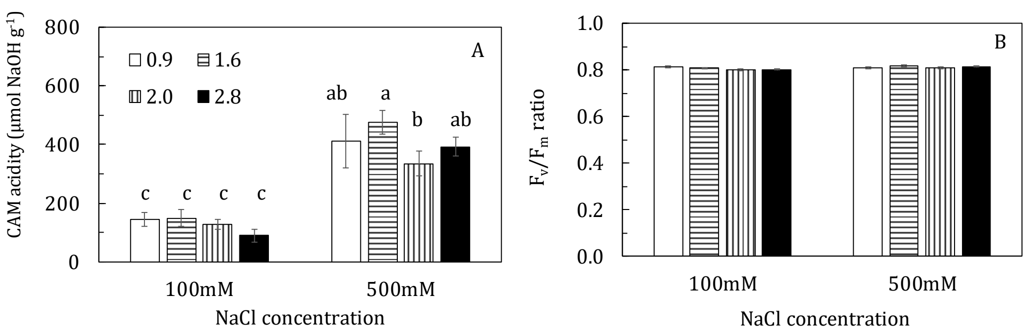

Figure 5 CAM acidity (A) and F_v/F_m ratio (B) of *M. crystallinum* grown under different R/B LED ratios at 100 and 500 NaCl concentrations for 14 days. Vertical bars represent the standard errors. Means with different letters are statistically different ($p < 0.05$; n=6 (A), n=8 (B)) as determined by LSD multiple comparison test.

Results of qP, ETR and NPQ measured under a PPFD of 281 μmol m^{-2} s^{-1}, which was close to the growth irradiance, are shown in Figure 6. *M. crystallinum* grown with 100 mM NaCl under R/B 0.9 had significant higher qP (Figure 6A) and ETR (Figure 6B) than plants grown with 100 mM NaCl under R/B 2.8 and all plants with 500 mM NaCl. *M. crystallinum* had the highest NPQ with 500 mM NaCl under R/B 2.0 and 2.8 followed by R/B 0.9 and 1.6. Similar lowest NPQ values were obtained from plant grown with 100 mM NaCl regardless of R/B ratios (Figure 6C). In the CAM-inducible *M. crystalline* under high salt stress, lower qP and higher NPQ were observed by a number of researchers (Keiller et al., 1994, Broetto et al., 2007, Niewiadomska et al., 2011). Thus, NPQ can be used to estimate the degree of CAM induction. This was supported by the results of this study for the fact that *M. crystalline* grown with 500 mM NaCl had higher CAM acidity(Figure 5A) and NPQ (Figure 6C). However, this correlation was not observed in our previous study when chlorophyll fluorescence parameters were analysed under a low actinic light of 156 μmol m^{-2} s^{-1} (He and Qin, 2020) compared to a high PPFD of 281 μmol m^{-2} s^{-1} used for this study. It was also found that *M. crystalline* had higher NPQ under R/B 2.0 and 2.8 with 500 mM NaCl. However, the correlations among CAM induction, photosynthetic light use efficiency, light quality and salinity may dependent on the intensity of growth irradiance as photosynthetic capacity is reduced by the synergistic effects of salinity and high light (Broetto et al., 2007).

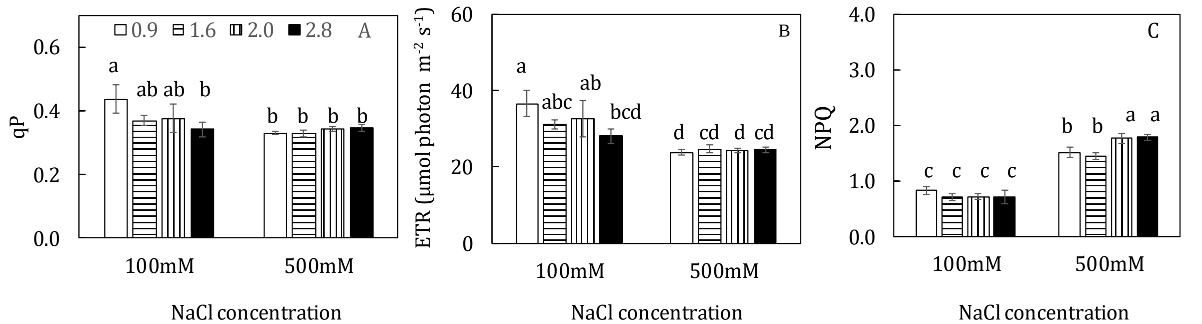

Figure 6 qP (A), ETR (B) and NPQ (C) measured at a PPFD of 281 μmol m^{-2} s^{-1} of *M. crystallinum* grown under different R/B LED ratios with 100 and 500 NaCl concentrations for 14 days. Vertical bars represent the standard errors. Means with different letters are statistically different ($p < 0.05$; n=4) as determined by LSD multiple comparison test.

CONCLUSIONS

M. crystallinum grown with 100 mM NaCl especially under high R/B 1.6, 2.0 and 2.8 had much higher shoot productivity and faster leaf growth compared to those plants grown with 500 mM NaCl. Regardless of R/B LED ratios, leaf succulence was higher in *M. crystallinum* grown with 100 mM NaCl than with 500 mM NaCl. Higher SLA in *M. crystallinum* plants grown with 100 mM NaCl under all R/B LED ratios could be due to their lower LDMC but high leaf water content. Induction and stimulation of CAM photosynthesis were observed in *M. crystallinum* grown with high salinity of 500 mM NaCl, especially under R/B 1.6. However, photoinhibition of photosynthesis did not occur in any plant as F_v/F_m ratios of all plants were greater than 0.8. All plants grown with 500 mM NaCl had higher CAM acidity and higher NPQ, especially under R/B 2.0 and 2.8. These results indicate that NPQ can be used to estimate the degree of CAM induction and LED quality had certain impacts on photosynthetic performance in *M. crystallinum* grown under different salinities. However, the correlations between CAM induction and photosynthetic light use efficiency under different LED quality and salinity may dependent on the intensity of growth irradiance which merits our further study.

ACKNOWLEDGEMENT

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