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***Methylobacterium oryzae* CBMB20 influences photosynthetic traits, volatile emission and ethylene metabolism in *Oryza sativa* genotypes grown in salt stress conditions**

Poulami Chatterjee¹, Arooran Kanagendran², Sandipan Samaddar¹, Leila Pazouki^{2,3}, Tong-Min Sa^{1,*}, Ülo Niinemets^{2,4,*}

¹Department of Environmental and Biological Chemistry, Chungbuk National University Cheongju, Chungbuk 28644, Republic of Korea

²Institute of Agricultural and Environmental Sciences, Estonian University of Life Sciences, Kreutzwaldi 1, Tartu 51006, Estonia

³Department of Biology, University of Louisville, Louisville, KY 40292, USA

⁴Estonian Academy of Sciences, Kohtu 6, 10130 Tallinn, Estonia

Abstract

The objective of this study was to analyze the impact of ACC (1-aminocyclopropane-1-carboxylate) deaminase producing *Methylobacterium oryzae* CBMB20 in acclimation of plant to salt stress by controlling photosynthetic characteristics and volatile emission in salt-sensitive (IR29) and moderately salt resistant (FL478) rice (*Oryza sativa* L.) cultivars. Saline levels of 50 mM and 100 mM NaCl with and without bacteria inoculation were applied, and the temporal changes in stress response and salinity resistance were assessed by monitoring photosynthetic characteristics, ACC accumulation, ACC oxidase activity (ACO), vacuolar H⁺ ATPase activity and volatile organic compound (VOC) emissions. Salt stress considerably reduced photosynthetic rate, stomatal conductance, PSII efficiency and vacuolar H⁺ ATPase activity, but it increased ACC accumulation, ACO activity, green leaf volatiles (GLV), mono- and sesquiterpenes, and other stress volatiles. These responses were enhanced with increasing salt stress and time. However, rice cultivars treated with CBMB20 showed improved plant vacuolar H⁺ ATPase activity, photosynthetic characteristics and decreased ACC accumulation, ACO activity and VOC emission. The bacteria-dependent changes were greater in the IR29 cultivar. These results indicate that decreasing photosynthesis and vacuolar H⁺ ATPase activity rates and increasing VOC emission rates in response to high salinity stress were effectively mitigated by *Methylobacterium oryzae* CBMB20 inoculation.

***Correspondence:** Tongmin Sa, Tel number: +82 43 261 2561; Fax: +82 43 271 5921, tomsa@chungbuk.ac.kr. **Co-correspondence:** Ülo Niinemets, Tel number: +372 534 57189, ylo.niinemets@emu.ee.

Author contributions

PC and TS: Conceptualization; PC: data curation; PC, AK, ÜN and TS: Formal analysis, original draft of the article; PC and SS: Visualization; PC, ÜN and TS: Funding acquisition and resources; PC, AK, SS, LP TS, and ÜN: review and editing

Conflict of Interest Statement

None.

Keywords

ACC deaminase; ACC oxidase; F_v/F_m ; Salt stress; Vacuolar H^+ ATPase; VOC

Introduction

Global agricultural productivity is largely affected by soil salinity, which impacts 1 billion hectares of land in nearly 100 countries (Ivushkin et al. 2018). The problem of reduced crop productivity due to salinity is constantly increasing as land degradation due to salinity is increased at the rate of 2 million hectares per year (Ivushkin et al. 2018). Enhanced salinity reduces the osmotic potential of the soil water, resulting in impaired rates of primary and secondary metabolic activity (Ahuja et al. 2010; Sanoubar et al. 2016), disruption of cell membranes, reduction in essential ion uptake, nutrient imbalance, and reduced stomatal conductance (Gupta and Huang 2014) in plants. In addition, excessive accumulation of Na^+ ions in the chloroplasts and reduced osmotic potential of mesophyll cells ultimately damage the photosystem II (PS II) and photosynthetic electron transport, causing a sustained reduction in photosynthesis (Sudhir and Murthy 2004; Sanoubar et al. 2016).

Plants produce an array of volatiles constitutively and under stress (Niinemets 2010; Copolovici and Niinemets 2016). Various abiotic stresses have been shown to increase VOC emission in different plant species, e.g. acute heat shock in *Solanum lycopersicum* (Pazouki et al. 2016), and flooding in three temperate deciduous woody species (Copolovici and Niinemets 2010). These studies have demonstrated that, the emission of volatile scales up with the severity of stress and thus, monitoring stress volatile emissions can provide a promising tool for non-invasive characterization of stress.

GLVs are characteristic stress-induced volatiles, biosynthesized by lipoxygenases from free polyunsaturated fatty acids (Feussner and Wasternack 2002). However, different stresses often activate 2-C-methyl-D-erythritol 4-phosphate/1-deoxy-D-xylulose 5-phosphate (MEP/DOXP) and mevalonate (MVA) pathways of isoprenoid synthesis, leading to the biosynthesis of mono- and sesquiterpenes (Pazouki and Niinemets 2016). In addition to GLV, and mono- and sesquiterpenes, various stresses often lead to enhanced emissions of short-chained oxygenated volatile organic compounds (OVOC), saturated aldehydes, volatile products of geranyl-geranyl diphosphate pathway (GGDP) and benzenoids (Kask et al. 2016; Kanagendran et al. 2017, 2018). Emission of the stress hormone ethylene is also triggered by various abiotic stresses including salinity stress and thus, ethylene emissions are also of high diagnostic value (Madhaiyan et al. 2006a; Yim et al. 2013). Ethylene emissions often scale with the severity of stress or the degree of plant resistance to stress (Zapata et al. 2003). The emission is regulated by ACC (Arimura et al. 2002). Therefore, monitoring changes in volatile emission rates and the factors controlling ethylene emission under salinity can provide quantitative insight into the stress severity as well as induction of various secondary metabolic pathways throughout the stress propagation and recovery phases.

Rice (*Oryza sativa* L.) is cultivated throughout the tropical regions and feeds more than half of the world's population (Seck et al. 2012). Rice is a salt-sensitive crop that is adversely

affected by even mild salinity stress (Yeo et al. 1999). Therefore, it is widely considered as a model plant for studies related to salinity stress (Dionisio-Sese and Tobita 1998).

Plants species have developed several strategies to cope with abiotic stresses, including reduction in water loss through stomata, changes in metabolism, control of ionic and osmotic stress, reduction in stress elicited reactive oxygen species (ROS) generation (Abogadallah 2010) and increased activity of vacuolar H⁺ ATPases (Ahuja et al. 2010). Maintenance of H⁺ ATPase activity is required to retain the high concentrations of K⁺ and lower concentrations of Na⁺ in plants (Zhu 2003). H⁺ ATPase acts as cellular house keeper, by establishing the electrochemical H⁺ gradient (Low et al. 1996). However, there is evidence that the constitutive level of salinity stress protection mechanisms and induction of these mechanisms under salinity is very limited in rice (Yeo et al. 1999). Indeed, previous studies have even indicated that salinity could even reduce H⁺ ATPase activity in rice (Chatterjee et al. 2018). There are several approaches to alleviate salinity stress in plants such as including cation foliar application (Sultana et al. 2001), selective breeding for salinity resistant lines, gene transformation for improving salinity resistance, and using plant growth promoting bacteria (PGPB) (Deinlein et al. 2014; Gupta and Huang 2014). Several reports have indicated the positive impact of PGPB on the growth and development of plants, especially under different stresses such as drought and salinity (Siddikee et al. 2011; Yim et al. 2013; Timmusk et al. 2014; Chatterjee et al. 2017), and among different options, using PGPB is considered as the potentially cost-effective and economically feasible strategy (Timmusk et al. 2014). Wang et al. (2016) reported that inoculation of ACC deaminase bacteria improved photosynthetic characteristics in salt stressed Pea plants. In fact, the ability of ACC deaminase producing bacteria (isolated from desert) to decrease the transcript levels of ion transport genes in *Arabidopsis thaliana* further supported the fact that the enhanced stress tolerance potential of ACC deaminase containing bacteria under salinity stress (Eida et al. 2019). There are very few studies evaluating the efficiency of PGPB in improving stress resistance by assessing time-dependent changes in VOC emission and photosynthetic characteristics (Timmusk et al. 2014; Matsuoka et al. 2016). In the case of ACC deaminase-harboring PGPB, it is suggested that improvement in stress resistance is associated with lack of hypersensitive ethylene response (Madhaiyan et al. 2007a, b).

The present study was undertaken to identify the potential role of *M. oryzae* CBMB20 (Ref seq ID: NZ_CP003811.1) in improving salinity stress through temporal modifications in photosynthetic characteristics, vacuolar H⁺ ATPase activity, ACC accumulation, ACC oxidase activity (ACO), and stress volatiles. *Methylobacterium oryzae* CBMB20 is a pink-pigmented ACC deaminase producing facultative methylotroph that has been isolated from stem surface of rice (Madhaiyan et al. 2007a; Kwak et al. 2014). *Methylobacterium oryzae* CBMB20 colonizes inside the plant parts and significantly promotes plant growth (Madhaiyan et al. 2010; Lee et al. 2011). Previous studies have suggested that ACC deaminase producing *M. oryzae* CBMB20 mainly improves plant growth and productivity through enhanced nutrient accumulation (Madhaiyan et al. 2010) and increased plant indole acetic acid and cytokinin production (Madhaiyan et al. 2006a; Madhaiyan et al. 2006b; Madhaiyan et al. 2007b).

In this study, we assessed time-dependent variations in (i) photosynthetic characteristics, (ii) vacuolar H⁺ ATPase activity, ACC accumulation, and ACC oxidase activity (ACO), and (iii) stress volatile emissions, including GLVs, mono- and sesquiterpenes, saturated aldehydes, OVOC, GGDP pathway volatiles, and benzenoids from salt sensitive (IR29) and salt tolerant (FL478) rice cultivars in response to combined application of salinity differing in severity (50 and 100 mM NaCl) and *M. oryzae* inoculation throughout the salt-stressed period.

We hypothesized that (i) *M. oryzae* CBMB20 inoculation increases foliage photosynthetic characteristics; that it (ii) increases vacuolar H⁺ ATPase activity, and decreases ACC accumulation and ACO activity; and that it (iii) suppresses stress volatile emission throughout the salt-stressed phase for both rice genotypes, (iv) the interactive effect of salt and bacteria are more effective with the increasing time and that (v) all these changes are more prominent for salt-sensitive genotype IR29 than for moderately salt-resistant genotype FL478.

Materials and methods

Plant materials and growth conditions

Seeds of moderately salt tolerant (FL478) and salt sensitive (IR29) rice cultivars (*O. sativa* L.) obtained from Rural Development Administration (RDA, Suwon, South Korea), initially developed at International Rice Research Institute (IRRI, Manila, Philippines) were used in this study. Sodium hypochlorite (NaOCl) solution (1 %) was used for seed surface sterilization. This treatment was followed by several washes in sterile distilled water (250 mL per wash), and the seeds were further dried overnight in a laminar flow cabinet. The surface sterilized seeds were evenly placed on wet paper towel kept in Petri dishes (max. ~ fifty seeds per Petri dish) and then left for germination (Lee et al. 2006). Seed germination was carried out by incubating the seeds in dark for three days and then they were kept in Percival AR-95 HIL growth chamber (CLF Plant Climatics GmbH, Wertingen, Germany), under the standard environmental conditions: light intensity of 300-400 $\mu\text{mol m}^{-2} \text{s}^{-1}$ for 12 h photoperiod, day and night temperatures of 22/18 °C, and relative air humidity of 60%. After one week, the germinated seedlings were transplanted into 350 mL pots filled with commercial potting mixture (Biolan Oy, Kauttua, Finland) and fertilized with a commercial liquid fertilizer (NPK 5:5:6 and micronutrients B- 0.01 %, Cu- 0.03 %, Fe- 0.06 %, Mn- 0.028 %, Zn- 0.007 %) according to the recommended dosage. One week old seedlings were translocated into a plant growth room, where they were grown under a light intensity of 400-500 $\mu\text{mol m}^{-2} \text{s}^{-1}$ provided by HPI-T Plus 400 W metal halide lamps (Philips) for 12 h photoperiod, day / night temperature of 28 / 25 °C, ambient CO₂ concentration of 380-400 ppm, and relative air humidity of 60-70 % till the experiment was completed. The plants were watered for optimum soil moisture without any flooding or drought conditions throughout the experimental period.

Bacterial inoculation

ACC deaminase producing *M. oryzae* CBMB20 (Ref. Seq: NZ_CP003811.1) was grown in ammonium mineral salts (AMS) minimal broth medium, supplied with 0.5 % sodium succinate as the sole carbon source at 25 °C. Bacterial inoculation in rice rhizosphere was

conducted at 7 and 14 days after sowing seeds by adding 10 mL of the bacterial suspension (prepared using 0.03M $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$) (10^8 CFU mL^{-1} at A_{600}) closer to the root zone of seedlings. In addition, foliage inoculation by bacteria was also carried out according to Esitken et al. (2010). Non-inoculated plants were treated with sterile 0.03M $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ solution (carrier solution) at the time of bacterial inoculation.

Salt stress application

Twenty one day-old rice plants were treated with two levels of salt stress, 50 mM and 100 mM NaCl solution. In the case of 50 mM salt stress, 10 mL of 50 mM of NaCl solution was applied to each plant at once. However, in the case of 100 mM salt stress, 5 mL of 100 mM NaCl solution was applied at two day-interval to each plant to prevent severe osmotic shock. The measurements for photosynthetic traits, stress volatile emission, vacuolar H^+ ATPase activity, ACC accumulation, and ACC oxidase (ACO) activity were measured upon achieving desired salt concentration in each case separately (as we applied 50 mM once and 100 mM in two intervals, the 1st, 5th, 10th day of measurement are different for each treatment). Non-treated and bacteria treated control plants were well-watered throughout the experimental period.

Leaf gas-exchange and volatile measurement setup and estimation of leaf area

A 1.2 L cylindrical doubled-walled glass chamber was used for gas-exchange measurements and volatile gas collection. Distilled water of 25 °C was continuously circulated between the layers of glass chamber walls to maintain a stable chamber temperature. The air temperature was monitored by a thermistor (NTC thermistor, model ACC- 001, RTI Electronics, Inc., St. Anaheim, CA, USA), whereas leaf temperature was monitored by a thermocouple attached to the lower sides of leaf. Illumination was provided by four 50 W halogen lamps, and light intensity at the leaf surface was set at 700-800 $\mu\text{mol m}^{-2} \text{s}^{-1}$. A fan enclosed in the chamber was used to maintain high air turbulence to minimize leaf boundary layer resistance. In addition, charcoal-filtered ozone-free ambient air was supplied to the chamber. CO_2 concentration in the air inlet was $380 \pm 20 \mu\text{mol mol}^{-1}$. The measurements were conducted at an air humidity of 60-70 %, and this humidity level was maintained by a custom-made humidifier.

Teflon® and stainless steel tubing and connections were used throughout the gas-exchange system. The chamber gas lines were regularly switched between the reference (ingoing air) and measurement (outgoing air) modes to take gas-exchange measurements. An infrared dual-channel gas analyzer (CIRAS II, PP-systems, Amesbury, MA, USA), was used to measure changes in CO_2 and H_2O concentrations between the chamber reference and measurement modes. Further details about the gas-exchange system can be found in Copolovici and Niinemets (2010).

Fully mature leaves were used for the measurements. Gas-exchange and VOC emission collection were carried out at days 1, 5 and 10 days since the application of salt stress. After leaf enclosure, the standard environmental conditions were established, and gas exchange rates were recorded once they reached the steady state (in ca. 15-20 min after leaf enclosure), and volatile collection started thereafter. The leaves enclosed in the gas-exchange

chamber were scanned at 300 dpi to estimate the leaf area using a custom-made software. Foliage net assimilation rate (A) and stomatal conductance to water vapour (g_s) were calculated according to von Caemmerer and Farquhar (1981).

Chlorophyll fluorescence measurements

After completion of gas-exchange measurements and VOC collection, the dark-adapted chlorophyll fluorescence yield (10 min darkening, F_v/F_m) was estimated with an imaging PAM fluorometer (Walz IMAG-MIN/B, Walz GmbH, Effeltrich, Germany) using a saturating pulse ($7000 \mu\text{mol m}^{-2} \text{s}^{-1}$ for 1 s) of blue light was used to measure the maximum dark-adapted fluorescence yield, F_m .

VOC sampling, and analysis by gas chromatography-mass spectrometry (GC-MS)

VOCs were collected onto multi-bed stainless steel adsorbent cartridges filled with layers of Carbotrap adsorbents for the quantitative adsorption of C3-C18 volatiles (Kännaste et al. 2014 for detail description). A portable suction pump 210-1003 MTX (SKC Inc., Houston, TX, USA) was used, and volatiles were sampled at a suction flow at air suction rate of 200 mL min^{-1} for 20 min. In addition, volatiles were also collected from the chamber in the absence of leaves to estimate net leaf VOC emission from leaves.

The adsorbent cartridges were analyzed with a combined Shimadzu TD20 automated cartridge desorber and Shimadzu 2010 Plus GC-MS system (Shimadzu Corporation, Kyoto, Japan) with Zebron ZB-624 fused silica capillary column ($0.32 \text{ mm i.d.} \times 60 \text{ m}$, $1.8 \mu\text{m}$ film thickness), (Phenomenex, Torrance, CA, USA) for GLV, monoterpenes, sesquiterpenes, saturated aldehydes short-chained oxygenated volatiles (OVOC), geranylgeranyl diphosphate pathway volatiles (GGDP volatiles), and benzenoids. The compounds were identified based on pure standards (GC purity, Sigma-Aldrich, St. Louis, MO, USA) and NIST 05 spectral library. A detailed description of GC-MS analysis and compound identification can be found in Niinemets et al. (2011), and Kännaste et al. (2014). The emission rates were calculated according to Niinemets et al. (2011).

Estimation of foliar ACC accumulation and ACC oxidase activity

The ACC accumulation and ACC oxidase activity were estimated from the leaves of FL478 and IR29 rice cultivars according to the protocol described by Lizada and Yang (1979), and Yim et al. (2013). Briefly, one gram of fresh leaves were ground in liquid nitrogen. Each sample was dissolved in 5 mL of 80 % methanol containing butylated hydroxyl toluene as an antioxidant (BHT, 2 mg L^{-1}) and incubated for 45 min. Then the samples were centrifuged at $2,000 g$ for 15 min. The pellet was re-suspended in 4 mL of methanol and then centrifuged at $2,000 g$ for 15 min. The combined supernatant was extracted and then dried using rotary vacuum evaporator (Eyela world, Tokyo, Japan).

To estimate ACC accumulation, the dried residues were re-suspended in 2 mL of distilled water. The mixture was left undisturbed, until the upper aqueous layer was separated itself after extracting with dichloromethane mixed with 0.1 mL HgCl_2 (80 mM) in a test tube. Thereafter, the test tubes were sealed with a rubber septum and then, 0.2 mL of alkaline NaOCl solution (obtained by mixing 40 mL NaOH , $80 \text{ mL } 12.5 \% \text{ NaOCl}$ solution, and 30

mL distilled water) was injected into each test tube. The tubes were shaken and incubated for 8 min and concentration of the released ethylene was analyzed by gas chromatograph using the Poropak-Q column (dsCHROM 6200, Donam Instruments Inc., Republic of Korea). The ACC accumulation was estimated as average of three replicate samples. For calibration, replicate samples containing internal standards of ACC were used as explained in Yim et al. (2013).

To estimate ACC oxidase (ACO) activity leaf samples were ground in liquid nitrogen. The leaf sample was homogenized in 2 mL g⁻¹ of extraction buffer (comprised of 100 mM Tris-HCl (pH 7.2), 10 % (w/v) glycerol, and 30 mM sodium ascorbate) and then centrifuged at 25,704 g for 15 min at 4°C. The ACO enzyme activity was estimated from 1.5 mL of supernatant in a solution consisting of 50 mM FeSO₄, 1 mM ACC, and 5 % (v/v) CO₂, kept in 10 mL screw-cap tubes fitted with a Teflon® coated septum. The sample was incubated at 30 °C for 15 min and the quantity of ethylene released into the headspace was estimated by gas chromatograph as explained above by Yim et al. (2013).

Vacuolar H⁺ ATPase activity of rice leaves

Vacuolar H⁺ ATPase activity was determined according to the release of pyrophosphate in ATP hydrolysis. The plant protein was extracted according to Wang et al. (2003) and measured by Bradford (1976) using bovine serum albumin as a standard. Five hundred microliter of reaction mixture was prepared containing: 15 µg of protein in 50 mM KCl, 30 mM bis-tris-propane (pH = 8), 150 µM gramicidin D, 0.1 mM NaMoO₄, 1 mM NaN₃, 0.1 mM EDTA with bis-tris-propane (pH = 8), 1 mM ATP, 0.1 mM Na₃VO₄. Ten microliter of 100 mM MgSO₄ was added to initiate the reaction. The reaction mixture was incubated at 35 °C for 20 minutes. 1.5 mL of stop solution (containing six portions of 0.42 % ammonium molybdate in 1 N H₂SO₄ solution and one part of 10 % ascorbic acid) was used to stop the reaction. Pi release was measured at spectrophotometrically at 800 nm (Carystinos et al. 1995). The ATPase activity was calculated in relation to total protein concentration.

Data analyses

In this study, there were six different treatments (0 mM NaCl, Bacteria only, 50 mM NaCl, 50 mM NaCl + Bacteria, 100 mM NaCl, 100 mM NaCl + Bacteria) applied for three different sampling-time points (1, 5, and 10 days after treatments) and three individual plants for three replicates (for each time point, treatment, and for each cultivar). In this experiment, a single and different plant was used as single replicate for each treatment at each time point. As we worked with two cultivars, a total of 108 individual plants was used in this study.

The data were evaluated for normality of distributions and homogeneity of variances to comply with parametric test assumptions and then the data were log-transformed, when needed. Generalized linear models (GLM) based on maximum likelihood model fitting were applied to estimate the statistical impact of individual and interactive effects of salt, bacteria, and time after treatment on photosynthesis characteristics, volatile emissions, ACC accumulation, ACO activity, and vacuolar H⁺ ATPase activity. In principle, salt, bacteria, and sampling times after treatments are co-variables. Therefore, application of GLM is considered as the most appropriate statistical model in this study (Chatterjee et al. 2018). As

GLM was done by maximum likelihood method, this is much less-prone to problems due to non-normality of data. The results of detailed statistical analysis of GLM have been provided in the table S1, where main and key interaction effects of salt, bacteria, and sampling times after treatments on photosynthetic characteristics, stress volatile emissions, ACC accumulation, ACO activity, and vacuolar H⁺ ATPase activity for both the cultivars were interpreted. GLM was conducted with SPSS v.23 (IBM SPSS, Chicago, IL, USA). Data plots were made with SigmaPlot 12.5 (Systat Software Inc, San Jose, CA, USA). Pearson correlation matrix among total volatile emission rates, ACO activity and ACC accumulation in both rice cultivars was generated with R studio 1.0.143 (Wei and Simko 2013). All statistical effects were considered significant at $P < 0.05$.

Results

Impact of salt stress and bacteria on foliage photosynthetic characteristics

The maximum dark-adapted photosystem II (PS II) quantum yield as estimated by chlorophyll fluorescence (F_v/F_m) was reduced by 50 and 100 mM NaCl treatments in both rice cultivars (Fig. 1 a, d; $P < 0.001$). However, a sustained reduction was observed in plants exposed to 100 mM NaCl treatment with more severe effect in the salt-sensitive cultivar IR29 compared to moderately salt resistant FL478 (Fig. 1). Similarly, separate salt treatments and combined salt and bacteria treatments declined net assimilation rate (A) and stomatal conductance to water vapor (g_s) (Table S1 and Fig. 1 b-f; $P < 0.001$). In all cases, changes in A were primarily correlated with the changes in g_s . The reduction in F_v/F_m , A , and g_s was lower for the plants inoculated with bacteria and subsequently treated with salt treatments than for the plants treated with salt treatments alone. In fact, bacterial inoculation alone enhanced photosynthetic characteristics (Fig. 1). In all cases, the changes of photosynthetic characteristics were more pronounced in the salt-sensitive cultivar IR29 than in the moderately salt resistant cultivar FL478 (Table S1 and Fig. 1 b-f).

Effects of salt and bacteria on green leaf volatile (GLV) emission

Among induced GLV, only hexanal and pentanal were observed. The emissions of hexanal were greater than the emissions of pentanal in both rice cultivars (Table S1, S2 and S3). The emission of total GLV by control leaves was in the range of ca 0.017 – 0.028 nmol m⁻² s⁻¹ for both cultivars. However, 100 mM salt treatment elicited GLV emission to a level of 0.344 nmol m⁻² s⁻¹ for IR29 and to 0.253 nmol m⁻² s⁻¹ for FL478 at day 1 after treatments and the emission was increased progressively with the time from the treatment (Table S1 and Fig. 2a and d; $P < 0.001$ for salt and combined salt and bacterial treatments). In all cases, GLV emission scaled with the severity of stress applications (Fig. 2a and d). Furthermore, bacterial inoculation suppressed GLV emission rate in a time-dependent manner, by 86 % for FL478 and 81 % for IR29 on the 10th day since the application of 100 mM salt stress.

Emission of mono- and sesquiterpenes in response to salt stress and bacteria inoculation

Low rates of monoterpene emission were observed under non-stressed conditions, ranging between ca 0.048 – 0.144 nmol m⁻² s⁻¹ for FL478 and 0.059 – 0.103 nmol m⁻² s⁻¹ for IR29 (Fig. 2b, e). The individual monoterpenes observed were α -pinene, β -myrcene, β -phellandrene, β -pinene, camphene, limonene, ³-carene and 1,8-cineole in both rice

cultivars (Table S2 and S3). In FL478, the bouquet of monoterpenes was dominated by 1,8-cineole followed by α -pinene, and in IR29, it was dominated by 1,8-cineole followed by β -carene and α -pinene (Table S2 and S3). Salt treatment alone increased monoterpene emission rates in a time-dependent manner in both cultivars, whereas the emission maxima reached were $0.608 \text{ nmol m}^{-2} \text{ s}^{-1}$ for 50 mM NaCl and $1.66 \text{ nmol m}^{-2} \text{ s}^{-1}$ for 100 mM NaCl for IR29. Inoculation by bacteria drastically reduced total monoterpene emission rate (Fig. 2b, e). For the severe salt treatment of 100 mM, at day 1, 55% reduction was observed for both cultivars, whereas at day 10, 60% reduction was observed for FL478 and 78% reduction for IR29 (Table S1; $P < 0.001$; Fig. 2b, e).

The salt-elicited sesquiterpene emissions were dominated by (*E*)- β -caryophyllene in both rice cultivars. Elicitation of longifolene and viridiflorene emissions occurred only upon 100 mM NaCl salt treatment (Table S2 and S3). *Methylobacterium oryzae* CBMB20 inoculation reduced sesquiterpene emissions in a time-dependent way (Table S3 and Fig. 2c, f) similarly to monoterpene emission in both rice cultivars. The effect of bacterial inoculation was stronger for total sesquiterpene emission for 100 mM NaCl treatment, where total sesquiterpene emissions was decreased by 75 % in FL478 and 53 % in IR29 at day 10 after application of the salt treatment (Fig. 2c, f). In all cases, sesquiterpene emission was positively associated with the severity of salinity stress ($P < 0.001$; Table S1 and Fig. 2c, f).

Emission rates of saturated aldehydes, OVOC, GGDP volatiles, and benzenoids in response to salt stress and bacterial inoculation

Four long-chain saturated aldehydes, heptanal, octanal, nonanal and decanal were observed in both rice cultivars (Table S2 and S3). Generally, application of salt stress increased total saturated aldehyde emission and increase was greater in IR29 than in FL478 (Table S1 and Fig. 3a, c; $P < 0.001$ for all treatments). Saturated aldehyde emissions after salt treatment were dominated by octanal in both cultivars (Fig. 3a, c). Inoculation by *M. oryzae* CBMB20 significantly reduced the emission of total and individual saturated aldehydes ($P < 0.001$) upon subsequent treatment with NaCl in both cultivars (Table S1, S2 and Table S3).

Among OVOC, both rice cultivars emitted acetaldehyde and acetone (Tables S2 and S3). Total OVOC emission rate of control leaves varied between $0.034 - 0.044 \text{ nmol m}^{-2} \text{ s}^{-1}$ for FL478 and $0.030 - 0.041 \text{ nmol m}^{-2} \text{ s}^{-1}$ for IR29, but all salt treatments exponentially increased total OVOC emission in a time-dependent manner (Fig. 3b, d; $P < 0.001$ for separate salt and combined salt and bacterial treatments) with a maximum emission detected at 100 mM salt treatment. Bacterial inoculation significantly reduced the OVOC emission from salt-stressed rice cultivars (Table S1; $P < 0.001$). However, in 100 mM salt-treated plants, OVOC emission was also enhanced in bacteria-treated plants, albeit less than in non-treated plants (Fig. 3b, d). Bacterial inoculation reduced salt-dependent elicitation of OVOC emission more in IR29 than in FL478 (Table S1 and Fig. 3b, d).

6-Methyl-5-hepten-2-one and (*E*)-geranyl acetone were the two GGDP pathway volatiles observed in this study. The GGDP pathway volatile emissions were dominated by (*E*)-geranyl acetone (ca. 57 % of total GGDP volatiles) after 1 day of 100 mM salt stress in FL478 (Tables S2 and S3). Separate NaCl treatment and combined NaCl and bacterial treatment significantly increased GGDP pathway volatile emissions, but the enhancement

was much less in bacteria-treated leaves ($P < 0.001$ for separate NaCl, and combined NaCl and bacterial treatment; Table S1 and Fig. 4a, c). At day 10 after the 100 mM salt treatment, the emission rate of GGDP volatiles was increased by 70 times for IR29 and 20 times for FL478 (Fig. 4a, c).

Three compounds emitted under the category of benzenoids, benzaldehyde, *p*-xylene and *o*-xylene were emitted from rice leaves, and the blend of benzenoids was dominated by benzaldehyde, followed by *p*-xylene (Table S2 and S3). Similar to the emission of other volatile classes, benzenoid emission scaled positively with the salinity stress severity (Table S1; $P < 0.001$ for all treatment effects). Salt-dependent changes in total benzenoid emission were the greater for IR29 than for FL478 and the bacterial inoculation reduced total benzenoid emission compared with non-inoculated plants (Fig. 4b, d).

Emission rates of total volatile emission in response to salt stress and bacterial inoculation from both the rice cultivars

The total VOC emission was lower in controls and lowest in only bacteria treated rice leaves. However, 50 mM and 100 mM salt exposure elicited the total volatile emission in both the rice cultivars and the emission was higher in salt sensitive, IR29 rice cultivar than the moderately salt tolerant FL478. Furthermore, the reduction in total VOC emission was observed in *M. oryzae* CBMB20 inoculated rice cultivars throughout the stressed period. The CBMB20 treated FL478 showed reduction in the emission by 22.3 % (in 50 mM + Bacteria), 54.09 % (in 100 mM + Bacteria) after 1 day, 42.52 % (in 50 mM + Bacteria), 60.65 % (in 100 mM + Bacteria) after 5 day, and 43.98 % (in 50 mM + Bacteria), 71.35 % (in 100 mM + Bacteria) after 10 day since the application of salt stress (Fig. 2-4).

However, CBMB20 inoculation more effectively reduced the total volatile emission in IR29 by 38.17 % (in 50 mM + Bacteria), 62 % (in 100 mM + Bacteria) after 1 day, 46.92 % (in 50 mM + Bacteria), 67.39 % (in 100 mM + Bacteria) after 5 day, and 66.70 % (in 50 mM + Bacteria), 80.12 % (in 100 mM + Bacteria) after 10 day since the application of salinity stress (Fig. 2-4). The bacterial population was also examined and the viability of bacteria under salt stress further supported the halo tolerance potential of bacteria (Table S4).

Changes in ACC quantity and ACO activity in response to salt stress and bacterial inoculation

To assess the relationship between ethylene emission and volatile emission, we analyzed foliage ACC accumulation and ACC oxidase (ACO) activity (Fig. 5a, d and Fig. 5b, e). In fact, ACC accumulation and ACO activity increased with the severity of stress applications (Fig. 5) and both characteristics were also positively correlated with total VOC emission through all the treatments and recovery times (Fig. 6). Application of 50 mM and 100 mM salt stress amplified ACC accumulation in FL478 by 3-folds and in IR29 by 5-folds at day 10 after the application of salt stress (Fig. 5a, d). Bacterial inoculation reduced the ACC accumulation by 29.1 % in IR29 and by 25.7 % in FL478 (Table S1 and Fig. 5a, d) and it significantly decreased ACO activity by 42 % and 30.8 % in IR29 at day 10 after 100 mM salt treatment (Table S1 and Fig. 5b, e).

Changes in H⁺ ATPase activity in *Oryza sativa* cv. FL478 and IR29 leaves in response to salt stress and bacterial inoculation

Vacuolar H⁺ ATPase activity was reduced following salinity treatment in both cultivars in a time-dependent manner (Table S1 and Fig. 5c, f; $P < 0.001$). The reduction was especially severe, 2.6-folds, in response to 100 mM salt stress in IR29 at day 10 (Table S1 and Fig. 5f). In FL478, vacuolar H⁺ ATPase activity was reduced by 46.4 % at day 10 after the application of 100 mM salinity stress (Fig. 5c). After bacterial treatment, the activity was improved in both non-stressed and salt-stressed plants. In bacteria-treated and 100 mM salt-stressed plants, the vacuolar H⁺ ATPase activity was enhanced by 3-folds in FL478 and 4.8-folds in IR29.

Discussion

Effects of salinity stress and inoculation by *M. oryzae* CBMB20 on foliage photosynthetic characteristics in *O. sativa*

In our study, the photosynthetic traits were continuously decreased after stress applications, implying that plant oxidative status was increased throughout the salt-stressed phase (Fig. 1). Generally, increased salinity above a threshold limit leads to Na⁺ ion accumulation in plant cells that is often associated with cell injury and retardation of growth and productivity or even cell death under severe stress due to enhanced oxidative stress and ROS accumulation (Chatterjee et al. 2017).

In addition, Na⁺ ion accumulation in chloroplasts impairs the photosynthetic machinery, particularly causing severe damage in reaction centers of photosystem II, leading to a significant reduction in F_v/F_m and suppression of whole chain linear electron transport activity causing a marked reduction in photosynthetic capacity (Basu et al. 1998; Kalaji et al. 2011). Our findings of reduced F_v/F_m in salt-stressed rice (Fig. 1a, d) are consistent with previous observations of reduced F_v/F_m in salt-stressed maize plants (Sheng et al. 2008), wheat (Zheng et al. 2009), and *Prunus dulcis* (Miller) D. Webb (Ranjbarfordoei et al. 2006).

Salt stress classically leads to decrease in net assimilation rate (Bongi and Loreto 1989; Arfan et al. 2007) as was observed in this study (Fig. 1b, e). The reduction in net assimilation rate can be primarily biochemical, associated with decreased rate of photosynthetic electron transport or reduced activity of ribulose-1,5-bisphosphate carboxylase/oxygenase (RUBISCO), the key enzyme in the Calvin cycle (Ghosh et al. 2001). Furthermore, enhanced accumulation of Na⁺ ions leads to upregulation of abscisic acid (ABA) biosynthesis, including ABA in stomatal guard cells causing stomatal closure that can further reduce net assimilation rate (Seeman and Critchley 1985). In our study, both biochemical (Fig. 1a, d) and stomatal (Fig. 1c, f) factors were responsible for the reduction in net assimilation rate in rice.

Inoculation of ACC deaminase-containing *M. oryzae* CBMB20 in both rice cultivars mitigated the effects of salinity stress on photosynthetic characteristics (Fig. 1), suggesting that bacteria inoculation preserved the integrity of photosynthetic machinery. PGPB improves photosynthesis by various mechanisms, for example, by increasing plant water use efficiency (WUE) (Mayak et al. 2004) and the enhanced accumulation of chlorophyll

content (Kim et al. 2010). Furthermore, *M. oryzae* CBMB20 is a pink pigmented bacteria, producing carotenoids (Madhaiyan et al. 2007a; Kwak et al. 2014), which act as strong antioxidant due to their strong redox properties (Gholizadeh 2012). Therefore, it suggests that bacterial inoculation contributed to the enhanced production of carotenoids in rice plants, associated with increased photosynthesis upon subsequent application of salinity stress (Sudhir and Murthy 2004). It is also possible that reduction of overall stress level by bacteria as explained below was directly responsible for less pronounced stomatal closure and biochemical limitations of photosynthesis. The exact mechanism of salt protective effects of *M. oryzae* CBMB20 needs to be studied further.

Elicitation of green leaf volatiles (GLV) under salinity stress

Acute salinity stress greatly enhances ROS accumulation and lipid peroxidation, ultimately eliciting the biosynthesis of GLVs (Arisz and Munnik 2011; Mostofa et al. 2015). Since the salt sensitivity or tolerance level varies across plant species and cultivars, it also translates to varying GLV biosynthesis and emission rates as observed in our study (Ashraf and Harris, 2004) (Fig. 2a, d).

In this study, we could not observe the emission of typical stress volatiles such as (*E*)-2-hexenal, (*Z*)-3-hexenol, 1-hexanol, and (*Z*)-3-hexenyl acetate and it could be associated with lower sampling frequency. Hexanal and pentanal were the dominant GLVs observed in this study, and the GLV blend was dominated by hexanal under salt stress (Table S2 and S3). In fact, Hexanal is generally produced by chloroplastic lipoxygenases through the oxygenation of polyunsaturated fatty acid (Mano et al. 2009) (Fig. 2a, d), but part of hexanal can also be produced according to the same pathway responsible for production of larger saturated aldehydes (Wildt et al. 2003). Similar to our observation, a dominant emission of hexanal was observed upon ozone stress in *Nicotiana tabacum* ‘Wisconsin’ (Kanagendran et al. 2017), heat stress in *Solanum lycopersicum* (Pazouki et al. 2016) and wounding in *Arabidopsis* leaves (Matsui et al. 2000).

It is known that *M. oryzae* CBMB20 inoculation reduces rice ethylene emission (Madhaiyan et al. 2007a), and this can also be inferred from reductions in metabolic precursor ACC and key enzyme ACO activity even under high salinity stress (Fig. 5a, d and Fig. 5b, e). This is relevant as ethylene synergizes the GLV emission in *Malus pumila* var. *domestica* (Cellini et al. 2016) and in *Zea mays* (Ruther and Kleier 2005). Thus, the reduction in GLV emission after *M. oryzae* CBMB20 inoculation (Fig. 2a, d) was likely associated with the negative effect of bacteria on ethylene emission, and accordingly, lack of hypersensitive oxidative response.

Changes in emission of foliage mono- and sesquiterpenes under salinity stress

Our results suggest that the specific blend of mono- and sesquiterpene emissions from non-stressed rice leaves might be due to the presence of nonspecific terpene storage pool in aerial parts of rice (Hinge et al. 2016). *De novo* biosynthesis of monoterpene is mainly dependent on the generation of NADPH and ATP, which is produced by the photosynthetic electron transport activity (Niinemets et al. 2002; Copolovici et al. 2011). However, we observed impaired photosynthetic machinery under salinity stress in our study (Fig. 1), thus the

substrate level control was likely not responsible for the enhanced monoterpene emissions upon salinity stress. However, it might be associated with the enzyme activity or regulation of genes in MEP pathway (Copolovici and Niinemets 2016), whereas the emission rate of the oxygenated monoterpene, 1,8-cineole, is regulated by stomatal conductance to some extent (Niinemets et al. 2002). Therefore we cannot exclude the possibility that 1,8-cineole emission was non-biochemically altered in our study (Table S1 and S2). In response to an increased salt concentration, enhanced terpene emissions in rice cultivars occurred (Fig. 2b, e). This increased foliage terpene emission is in line with the heat stress in *Quercus ilex* L. (Loreto et al. 1998) and in *Solanum lycopersicum* (Pazouki et al. 2016), although in both cases the emission has been decreased with the recovery time in both the cases. Furthermore, Tomescu et al. (2017) observed an increase in terpene emission, rate upon salt concentration gradient in soil water, agreement with our study. However, the progression of terpene emission with the increasing salt stress, even after 10 days likely related with the increasing oxidative stress upon salinity stress (Chatterjee et al. 2018). Upon *M. oryzae* CBMB20 treatment terpene emissions were also decreased associated with lowered ethylene emission under salt stress. Arimura et al. (2008) also reported that higher ethylene emission is positively associated with enhanced terpene emission in *Medicago truncatula* infested with *Spodoptera exigua* and increased benzenoid emission from *Petunia hybrida* 'Mitchell' (Schoorink et al. 2006) (Fig. 6). Furthermore, brown planthopper (*Nilaparvata lugens*) treatment in *O. sativa* plants (Lu et al. 2006) showed higher ethylene and volatile emission, which also give a cue regarding a coordinated relationship between ethylene and volatile biosynthesis.

Response of other stress-specific volatiles towards salinity stress

High salinity increased the emission of saturated aldehydes, OVOCs, GGDP volatiles and benzenoids in both rice cultivars (Fig. 3 and 4). Although the exact pathway of saturated aldehydes biosynthesis is unknown, but it has been reported that they partly share lipoxygenase or hydroperoxide lyase pathway (Feussner and Wasternack, 2002). This phenomenon is further confirmed by the circumstance that emission kinetics of saturated aldehyde was almost similar to emission kinetics of GLV in our study (Fig. 3a, c). In fact, lipid peroxidation or membrane damage due to hypersensitive reactions and ROS production under salinity stress often leads to enhanced biosynthesis of saturated aldehydes (Yang et al. 2006; Hu et al. 2009). In agreement with our findings, several abiotic stresses like heat stress (Kask et al. 2016), methyl jasmonate (Jiang et al. 2017), and combination of both ozone and wounding (Kanagendran et al. 2018) were shown to enhance saturated aldehyde emission, which was positively correlated with the emission of GLVs.

Acetaldehyde and acetone were the main OVOCs observed in our study (Table S1 and S2). Acetaldehyde is a primary product of lipoxygenase mediated oxidation of C18 fatty acids of the cell membranes (Blokina et al. 2003; Portillo-Estrada et al. 2015). So far, acetone biosynthesis is less studied (Karl et al. 2003). However, it is assumed that acetone is biosynthesized through cyanogenic pathway (Bracho-Nunez et al. 2011). Similar to acetaldehyde emission, acetone is also released in response to many abiotic stresses, including wounding in aspen (Portillo-Estrada et al. 2015) and ozone-wounding in eucalypt (Kanagendran et al. 2018). Formation of GGDP pathway volatile occurs as a result of

oxidative cleavage of carotenoid pathway products (Kask et al. 2016; Dudareva et al. 2013) or through three prenyl transferases (Schwab et al. 2008). In our study, GGDP pathway volatiles were elicited in response to salt treatments, and this elicitation was reduced in bacteria treated plants, suggesting that the salinity stress might have resulted in enhanced breakdown of carotenoids in entire carotenoid pathway and that this effect was remedied by bacteria (Gholizadeh 2012). On the other hand, carotenoids are not only important photosynthetic pigments, but also serve as important lipid-soluble antioxidants (Halliwell 1987; Kim et al. 2018), and greater emission of GGDP volatiles might also indicate a greater turnover of carotenoids in salt-stressed plants. In fact, a previous study indicated that salinity stress activated the carotenoid pathway in *Solanum lycopersicum* (Borghesi et al. 2011). In this study, benzenoid emissions were dominated by benzaldehyde, followed by α -xylene and p -xylene (Table S2, S3 and Fig. 4b, d). Benzenoids are common stress-induced volatiles, produced by the shikimate pathway (Pichersky et al. 2006; Gentner et al. 2014). Our finding is in line with the findings of increased benzenoid emission from rice plants fed by herbivores (Zhao et al. 2010) (Fig. 4b, d) and in response to ozone and wounding treatment in eucalypts (Kanagendran et al. 2018).

Production of carotenoids by *M. oryzae* CBMB20 might have also played a role in lowering OVOC, GGDP emission (oxidation product of carotenoids) (Kask et al. 2016) and the produced carotenoids protects against photo oxidative stress (Kwak et al. 2014).

Temporal modifications in ACC quantity, ACO activity, and H⁺ ATPase in response to salt and bacterial treatments

ACC deaminase-producing bacteria including *M. oryzae* CBMB20 causes hydrolytic cleavage of ACC, reducing the availability of ACC for the biosynthesis of ethylene. In addition, ACO uses ACC as a substrate to produce ethylene and thus, a reduction in both ACC accumulation and ACO activity due to PGPB inoculation leads to a decline in stress induced ethylene emission from plants (Madhaiyan et al. 2007b; 2010; Yim et al. 2013). Additionally Zulak and Bohlmann (2010) mentioned that induction of ACC accumulation and ACO activity leads to increased ethylene emission as observed in our study (Fig. 5a, d; Fig. 5b, e).

Stress-induced emission kinetics of ethylene consists of two characteristic peaks; the first peak of ethylene emission burst is known to rely on an endogenous pool of ACC and the second bigger peak of ethylene emission is associated with *de novo* synthesis of ACC (Yim et al. 2013; Glick 2014). Reduction of the magnitude of this second emission burst due to reduction in *de novo* synthesis of ACC caused by ACC deaminase lead to enhanced-stress resistance (Yim et al. 2013). However, abolishing the hypersensitive response alone would not be enough for improved tolerance under sustained salinity stress, and other factors such as bacterially-driven changes in H⁺ ATPase activity could play a role (Chatterjee et al. 2018).

There is limited information of how halotolerant ACC deaminase producing bacteria improve plant salinity resistance through altering ACC accumulation and ACO activity levels and vacuolar H⁺ ATPase activity (Chatterjee et al. 2017; Samaddar et al. 2018). A previous study demonstrated that vacuolar H⁺ ATPase activity was increased in bacteria

inoculated rice plants under salt stress, which was associated with a considerable improvement in salt stress resistance (Soontharapirakkul et al. 2010). Rice is a glycophyte and its salt tolerance is regulated by vacuolar H⁺ ATPase activity (Dabbous et al. 2017), which is located in the endomembrane system of the cells (Schumacher and Krebs 2010). In fact, vacuolar H⁺ ATPase activity maintains an acidic microenvironment in the endomembrane system, which is essentially required to carry out the primary biochemical processes of plant (Dettmer et al. 2006). However, as we observed in our study, high salinity impairs the vacuolar H⁺ ATPase activity (Fig. 5c and f) (Gronwald et al. 1990). Therefore, optimization of vacuolar H⁺ ATPase activity to the optimum level by *M. oryzae* CBMB20 upon salinity stress likely improved salinity resistance in rice (Zhu, 2003) (Fig. 5c, f).

In addition, enhanced stress resistance can be induced in plants by either removing Na⁺ from the cytosol (Schumacher and Krebs 2010) through translocating NADH: quinone oxidoreductase (Na (+)-NQR) (Chen et al. 2017) across the cell membrane or by biosynthesis of stress tolerance proteins by bacteria (Kwak et al. 2014). Furthermore, *M. oryzae* CBMB20 likely increased the nutrient accumulation capacity of plants, attributed with increased survival rate of plants under severe stress conditions, including salinity (Madhaiyan et al. 2007b). This, scenario may also have contributed to reduce total volatile emission in both the cultivars by bacteria (Fig. 2-4). Furthermore, *M. oryzae* CBMB20 possess high-affinity ATP-driven potassium transport (Kdp) system, ABC transporters, Na⁺/H⁺ antiporter, chaperone like proteins, pro (proline) proteins, glg (alpha-1,4 glucan phosphorylase) proteins and aqp (aquaporins) proteins and all these characteristics play major roles in improving salinity resistant in rice (descriptions of important halotolerant genes of bacteria has been provided in Kwak et al. 2014).

Conclusion

The overall study has revealed an important trait of plant growth promoting bacteria. Inoculation by *M. oryzae* CBMB20 improves salt resistance in plants indicated by decreased stress volatile emission, ACC accumulation, ACO activity, and increased photosynthesis and vacuolar H⁺ ATPase activity in response to subsequent exposure of plants to salinity stress. The reduction of ACC accumulation and ACC oxidase activity, associated with decreased GLV emission in salt-stressed plants previously inoculated with bacteria indicated that decreased pool of poly unsaturated fatty acids due to reduced leaf oxidative status. In addition, the reduction of mono- and sesquiterpenes likely indicated that the down regulation of terpene synthase gene expression. Taken together, these findings illustrated that the rate of improvement in photosynthesis and ATPase activity, and the degree of reduction in volatile emissions were significantly higher in salt-sensitive cultivar IR29, than in salt-tolerant cultivar, FL478, implying that cultivars with lower constitutive salt stress tolerance can be benefitted more from the application of ACC deaminase-containing bacteria. Moreover, future investigations should include the molecular level study of plant volatile and bacteria relationship to unfold a fundamental basis to fully understanding their interaction with plant hosts.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations

GLV	green leaf volatiles
VOC	volatile organic compounds
ACO	ACC oxidase
GGDP	geranyl-geranyl diphosphate pathway
OVOC	oxygenated volatile organic compounds
GC-MS	gas chromatography-mass spectrometry
MEP/DOXP	2-C-methyl-D-erythritol 4-phosphate/1-deoxy-D-xylulose 5-phosphate
MVA	mevalonate

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Main Conclusion

Inoculation of endophytic *Methylobacterium oryzae* CBMB20 in salt-stressed rice plants improves photosynthesis and reduces stress volatile emissions due to mellowing of ethylene-dependent responses and activating vacuolar H⁺-ATPase.

Fig. 1.

Average ($\pm$ SE) dark-adapted maximum quantum yield of photosystem II (PS II) estimated by chlorophyll fluorescence (F_v/F_m ; **a, d**), net assimilation rate (A ; **b, e**) and stomatal conductance to water vapor (g_s ; **c, f**) in the leaves of *Oryza sativa* L. moderately salt-tolerant cultivar FL478 (**a, b, c**) and salt-sensitive cultivar IR29 (**d, e, f**) at 1, 5, and 10 days following plant treatment with NaCl. The details of statistical analysis as in Table S1

Fig. 2.

Average foliage emission rate ($\pm$ SE) of total green leaf volatiles (GLV: **a, d**), total monoterpenes (**b, e**), and total sesquiterpenes (**c, f**) from the leaves of control, salt-stressed, bacteria-inoculated and bacteria-inoculated and salt-stressed leaves of *O. sativa* L. cv. FL478 (**a-c**) and IR29 (**d-f**) at days 1, 5, and 10 after application of salt stress. The details of statistical analysis as in Table S1

Fig. 3.

Average foliage emission rate ($\pm$ SE) of total saturated aldehydes (a, c), short-chained oxygenated volatile organic compounds (OVOC) (b, d) in *O. sativa* L. cv. FL478 (a, b) and IR29 (c, d). The details of statistical analysis as in Table S1

Fig. 4.

Average foliage emission rate ($\pm$ SE) of total geranyl geranyl diphosphate (GGDP) pathway (mainly the destruction products of carotenoids; **(a, c)**, and benzenoids **(b, d)** in *O. sativa* L. cv. FL478 **(a, b)** and IR29 **(c, d)**. The details of statistical analysis as in Table S1

Fig. 5.

Average ($\pm$ SE) ACC accumulation (a, d), ACC oxidase activity (b, e), and ATPase activity (c, f) in leaves of *O. sativa* L. cv. FL478 (a, b, c) and IR29 (d, e, f) at days 1, 5 and 10 since salt stress applications. The details of statistical analysis as in Table S1

Fig. 6. Correlation matrix representing Pearson's r values, corresponding to the size of the circle ($n = 16$). Blue to red color gradient denotes the positive to negative correlation among the studied variables mentioned in the figure.