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Inoculation of *Brevibacterium linens* RS16 in *Oryza sativa* genotypes enhanced salinity resistance: Impacts on photosynthetic traits and foliar volatile emissions

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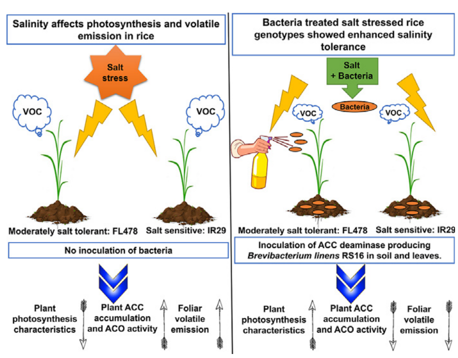
HIGHLIGHTS

- Salt stress reduces photosynthetic traits and increases stress volatile emission.
- Inoculation of *B. linens* RS16 enhances salinity resistance in rice cultivars.
- IR29 and FL478 rice cultivar differently responded to stress volatile emission.
- ACC accumulation and ACO activity reciprocally influence stress volatile emission.
- RS16 treatment decreases stress-induced foliar volatiles in *Oryza sativa* genotypes.

GRAPHICAL ABSTRACT



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ABSTRACT

The emission of volatiles in response to salt stress in rice cultivars has not been studied much to date. Studies addressing the regulation of stress induced volatile emission by halotolerant plant growth promoting bacteria containing ACC (1-aminocyclopropane-1-carboxylate) deaminase are also limited. The objective of the present study was to investigate the salt alleviation potential of bacteria by regulating photosynthetic characteristics and volatile emissions in rice cultivars, and to compare the effects of the bacteria inoculation and salt responses between two rice genotypes. The interactive effects of soil salinity (0, 50, and 100 mM NaCl) and inoculation with *Brevibacterium linens* RS16 on ACC accumulation, ACC oxidase activity, carbon assimilation and stress volatile emissions after stress application were studied in the moderately salt resistant (FL478) and the salt-sensitive (IR29) rice (*Oryza sativa* L.) cultivars. It was observed that salt stress reduced foliage photosynthetic rate, but induced foliage ACC accumulation, foliage ACC oxidase activity, and the emissions of all the major classes of volatile organic compounds (VOCs) including the lipoxygenase pathway volatiles, light-weight oxygenated volatiles, long-chained saturated aldehydes, benzenoids, geranylgeranyl diphosphate pathway products, and mono- and sesquiterpenes. All these characteristics scaled up quantitatively with increasing salt stress. The effects of salt stress were more pronounced in the salt-sensitive genotype IR29 compared to the moderately salt resistant

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VOCs

FL478 genotype. However, the bacterial inoculation significantly enhanced photosynthesis, and decreased ACC accumulation and the ACC oxidase activity, and VOC emissions both in control and salt-treated plants. Taken together, these results suggested that the ACC deaminase-containing *Brevibacterium linens* RS16 reduces the temporal regulation of VOC emissions and increases the plant physiological activity by reducing the availability of ethylene precursor ACC and the ACC oxidase activity under salt stress.

1. Introduction

Soil salinity is one of the key abiotic stresses that affect agricultural productivity throughout the world (Munns and Tester, 2008; Hasanuzzaman et al., 2013; Albaladejo et al., 2016; Oyiga et al., 2018). Nearly 2000 ha of fertile agricultural land degrades each day due to salinity, resulting in 20% of total land area in the world (Qadir et al., 2014). Salinity induces stress in plants by reducing the osmotic potential of soil water. Salt stress-driven accumulation of cellular Na^+ above a threshold level in chloroplasts causes tissue damage and increases the degree of chlorosis (Flowers et al., 2015). The salinity-induced, decrease in the osmotic potential of mesophyll cells has been shown to curb photosynthetic electron transport activities, ultimately leading to a significant reduction in photosynthesis (Chaves et al., 2009; Sudhir and Murthy, 2004).

Excessive ethylene emission is one of the first signals of stress response in plants (Madhaiyan et al., 2006; Yim et al., 2013), and it is dependent on the level of salt sensitivity or salt tolerance of the plant species (Zapata et al., 2003). However, in addition to ethylene, plants emit a plethora of VOCs (volatile organic compounds) (Copolovici and Niinemets, 2010; Copolovici et al., 2012; Copolovici et al., 2014; Mostofa et al., 2015; Pazouki et al., 2016; Carriero et al., 2016), which either provide for direct defense against the stress by serving as antioxidants (Vickers et al., 2009; Possell and Loreto, 2013) or indirect defense by serving as local and systemic signaling cues for intra- and interspecific communication in the plant community (War et al., 2012). Salt stress has been observed to exhibit a strong effect on volatile emissions (Loreto and Delfine, 2000; Zuo et al., 2012; Possell and Loreto, 2013), thus monitoring the volatile emissions under salinity can provide an illuminative insight into the severity of the stress as well as the induction of various secondary metabolic pathways with the progression of stress. While several studies have investigated the effects of salinity stress on the primary metabolism of plant and on the accumulation of the non-volatile secondary metabolites, there is limited understanding regarding the effects of salinity on the release of VOCs other than ethylene. GLVs (green leaf volatiles) are synthesized from free fatty acids released by phospholipases upon damage to cell membrane due to acute stress (Matsui, 2006; Gigot et al., 2010). Emission of the GLV is a ubiquitous response to various abiotic stresses, including salt stress in *Oryza sativa* (Mostofa et al., 2015), heat and cold shock in *Solanum lycopersicum* (Copolovici et al., 2012), heat shock in *Solanum lycopersicum* (Pazouki et al., 2016), flooding in *Alnus glutinosa*, *Populus tremula*, and *Quercus rubra* (Copolovici and Niinemets, 2010), drought in *Alnus glutinosa* (Copolovici et al., 2014), and ozone in *Betula pendula* (Carriero et al., 2016). Another class of plant VOCs, monoterpenes are biosynthesized in plastids through 2-*C*-methyl-D-erythritol 4-phosphate/1-deoxy-D-xylulose 5-phosphate pathway (MEP/DOXP pathway), whereas sesquiterpenes are biosynthesized in cytosol via mevalonate-dependent pathway (Rajabi Memari et al., 2013; Rosenkranz and Schnitzler, 2013). Monoterpene synthesis primarily depends on a continuous supply of NADPH and ATP generated via photosynthetic electron transport chain (Niinemets et al., 2002; Rasulov et al., 2016). Other than monoterpenes and sesquiterpenes, stress is also generally associated with the emissions of short-chained oxygenated volatile organic compounds (OVOCs), long-chained saturated aldehydes, benzenoids, and geranylgeranyl diphosphate (GGDP) pathway products (Kask et al., 2016; Kanagendran et al., 2017; Kanagendran et al., 2018).

Rice (*Oryza sativa* L.) is cultivated through warm temperate to tropical regions and is considered as staple food in Asia. Rice is generally, sensitive to salinity, which leads to a reduction in the growth, grain yield and development (Asch and Wopereis, 2001; Zeng et al., 2001). In recent years, various approaches (Deinlein et al., 2014) have been undertaken to assist plants to withstand salinity stress including the development of salt tolerant transgenic plants and also using plant growth-promoting bacteria (PGPB) to increase salinity tolerance in plants. Bacteria that contain 1-aminocyclopropane-1-carboxylate deaminase (ACC deaminase) have been demonstrated to decrease the emission of the plant stress hormone ethylene by reducing the level of ethylene precursor ACC, breaking it down to α-ketobutyrate and ammonia (Glick, 2014; Singh et al., 2015; Chatterjee et al., 2017). So far, only a small number of studies have been conducted to investigate the effects of PGPB on volatile emissions in stressed plants (Timmusk et al., 2014; Matsuoka et al., 2016). The mechanism through which a decrease in the ACC level leads to improved stress resistance is still not entirely understood. It is thought that the stress-dependent ethylene in plants occurs in two distinct peaks, one rapid ethylene burst that relies on an endogenous pool of ACC, followed a second longer-term, and typically higher emission burst that requires de novo synthesis of ACC (Pierik et al., 2006; Yim et al., 2013; Glick, 2014). Thus, suppression of the second ethylene emission burst by ACC deaminase-producing bacteria is suggested to be responsible for the improved stress resistance (Yim et al., 2013). So far, there is no information of how salt stress can effect volatile emissions in rice and how bacteria priming can change these emissions under salt stress. It is proposed that monitoring the VOC emission responses throughout the stress period, may support the potential action of the ACC deaminase-containing PGPB.

In the present study, two rice cultivars IR29 (salt-sensitive) and FL478 (moderately salt resistant) were used, to analyze the quantitative responses of carbon assimilation and stress volatile emissions to the combined effects of salinity and inoculation by the halotolerant PGPB *Brevibacterium linens* RS16, which was isolated from coastal soils in South Korea in a study, that aimed at isolating PGPBs to tolerate salt stress (Siddikee et al., 2010). This isolate can grow even at very salty conditions, >1.7 M NaCl, and has been shown to possess ACC deaminase activity and support plant growth under saline condition (Siddikee et al., 2011; Chatterjee et al., 2018).

In the present study, different concentrations of NaCl were applied in the potting mixture with and without inoculation with *B. linens* RS16 and examined (1) whether the bacterial inoculation improves foliage photosynthetic characteristics throughout the salt-stressed period; (2) how foliage stress volatile emissions respond to individual effects of NaCl and bacterial inoculation, and to the interactive effect of NaCl and bacteria after application of salt stress, until 10th day of the experiment; (3) how salt stress responses of photosynthetic characteristics and volatile emissions and the effect of inoculation with PGPB differ among two rice genotypes with varying degree of salt resistance. It was hypothesized that (1) salt stress affects foliage photosynthetic characteristics and causes the elicitation of stress volatile emissions in rice and all these modifications are more pronounced in the salt-sensitive rice genotype. (2) PGPB inoculation helped in the improvement of foliage photosynthetic characteristics and reduced volatile