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Exogenous application of L-histidine suppresses bacterial diseases and enhances ethylene production in rice seedlings

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Exogenous application of L-histidine enhances resistance to pathogens in tomato (*Solanum lycopersicum*) and *Arabidopsis thaliana* via activation of the ethylene (ET)-dependent signalling pathway. In this study, the efficacy of L-histidine for suppression of bacterial diseases in rice seedlings was investigated. Rice seeds were soaked in 10 mM L-histidine, 10

mM L-lysine, or distilled water (DW) as a control for 48 h at 25 °C to stimulate germination. Treated seeds were then vacuum-inoculated with *Burkholderia glumae* or *B. plantarii*. Seedling diseases caused by both of these bacterial pathogens were suppressed by treatment with L-histidine but not by treatment with L-lysine or DW. Expression of the ET-responsive defence-related gene, *OsGLP8-12*, was induced by treatment of seeds with L-histidine. As diseases were not suppressed in rice seedlings treated with L-histidine after vacuum-inoculation, pretreatment of rice seedlings with L-histidine before inoculation might activate the plant immune system. Indeed, ethylene production and the abundance of 1-aminocyclopropane-1-carboxylic acid (ACC) synthase 2 (*OsACS2*) transcript increased in healthy seedlings grown from rice seeds treated with L-histidine but not in those treated with DW. Furthermore, treatment of rice seeds with ACC, an ethylene precursor, suppressed bacterial rice seedling rot caused by *B. glumae* as effectively as did treatment with L-histidine, whereas treatment of rice seeds with aminooxyacetic acid (AOA), an inhibitor of ACC synthase, partially compromised disease suppression. Taken together, L-histidine seems to suppress bacterial rice seedling diseases via an ethylene-dependent resistance pathway.

Keywords: bacterial rice seedling disease, *Burkholderia glumae*, *Burkholderia plantarii*, ethylene, L-histidine

Introduction

The flexible defence system of host plants against a broad range of pathogens is positively or negatively regulated by signalling networks controlled by various endogenous signal molecules, including plant hormones (Robert-Seilaniantz *et al*., 2011; Pieterse *et al*., 2012; Vos *et al*., 2013; Gao *et al*., 2015). Some synthetic chemical compounds, so-called plant activators, mimic endogenous signal compounds that activate defence systems in plants and are used to protect against crop diseases in agricultural production (Leadbeater & Staub, 2014).

A natural plant activator AgrevoEX (Agrevo Co.), created from a yeast extract, has been commercialized in Japan and not only promotes growth and root formation but also suppresses the occurrence of plant diseases (Obara *et al*., 2007; Yoshida *et al*., 2010; Miyagawa & Nakaho, 2014). Seo *et al*. (2016) carried out studies to identify the effective component of AgrevoEX that controls plant diseases by analysing its ability to suppress bacterial wilt disease in tomato caused by *Ralstonia solanacearum*. The activity for inhibiting the development of wilt disease was found in the fraction with mol. wt. <3000, but not with mol. wt. >3000. The active fraction was fractionated further by chromatography on an

aminopropyl-based solid-phase extraction (SPE) and the fraction with the highest activity was subjected to HPLC. The most active fraction was purified further and a peak corresponding to the highest activity was analysed by magnetic resonance imaging. Resonances in ^{1}H and ^{13}C nuclear magnetic resonance (NMR) spectra for the peak were assigned to histidine. L-histidine, a natural form of histidine, was found to induce plant defences and thereby control the occurrence of wilt disease in tomato (Seo *et al*., 2016). Furthermore, L-lysine, another basic amino acid, also had disease suppressive activity; however, neither amino acids exhibited antibacterial activity.

Indeed, exogenous application of L-histidine, but not D-histidine, inhibited bacterial wilt disease in tomato and *Arabidopsis thaliana* and induced the expression of genes related to ethylene (ET) biosynthesis, ET-dependent signalling, and the production of ET in tomato plants (Seo *et al*., 2016). Furthermore, L-histidine-induced resistance to *R. solanacearum* was compromised in the *A. thaliana ein3-1* mutant, in which ET-mediated signalling is impaired (Seo *et al*., 2016). In addition to suppression of bacterial wilt disease in tomato treated with AgrevoEX, resistance to the fungal pathogen *Botrytis cinerea*, which also requires ET biosynthesis or signalling, was induced in *A. thaliana* by exogenously applied L-histidine (Seo *et al*., 2016).

Except for L-histidine, the potential of various other amino acids and their metabolites for control of diseases in crops has been reported. Methionine significantly decreased the severity of citrus bacterial canker caused by *Xanthomonas citri* subsp. *citri* (Xcc; Hasabi *et al*., 2014). Exogenous treatment of rice leaves with proteinogenic amino acids induced systemic resistance against rice blast fungus (Kadotani *et al*., 2016). Furthermore, amino acid metabolic pathways in plants are probably involved in the regulation of the plant immune system (Stuttmann *et al*., 2011; Seifi *et al*., 2013; Zeier, 2013; Fagard *et al*., 2014). For example, glutamate metabolism plays a key role in plant defence against pathogens (Seifi *et al*., 2013). Proline and pyrroline-5-carboxylate metabolism also function in plant defence against invading pathogens (Qamar *et al*., 2015). Furthermore, amino acid imbalances associated with homoserine or threonine accumulation elevated plant immunity to oomycete pathogens (Zeier, 2013). Interestingly, perturbations in amino acid homeostasis render mutant plants unsuitable as an infection host for *Hyaloperonospora arabidopsidis* (Stuttmann *et al*., 2011).

In contrast, conclusive studies of the disease suppressive activity of AgrevoEX and yeast extract, which is the main component of AgrevoEX, have been limited to suppression of anthracnose caused by *Colletotrichum orbiculare* in *Cucumis sativus* (Miyagawa &

Nakaho, 2014) and grey blight caused by *Pestalotiopsis longiseta* in *Camellia sinensis* (Yoshida *et al*., 2010). However, from the viewpoint of disease control in agricultural production, further expansion of the practical use of AgrevoEX against other diseases would be beneficial. Therefore, an improved understanding of the molecular mechanism(s) by which AgrevoEX might suppress other diseases in other plant species is required. In this study, the potential of L-histidine and L-lysine treatment to suppress rice bacterial seedling rot disease and rice bacterial seedling damping-off disease, which are caused by *Burkholderia glumae* and *B. plantarii*, respectively, and the possible role of ET in the disease suppressive activity of L-histidine were investigated.

Materials and methods

Plant and pathogen

Rice seeds (*Oryza sativa* 'Koshihikari') were incubated in distilled water at 60 °C for 10 min to sterilize seed surfaces and used for all experiments. *Burkholderia glumae* (MAFF302746; Uematsu *et al*., 1976) and *B. plantarii* (MAFF302475; Azegami *et al.*, 1987) were supplied from the Genetic Resources Center, NARO, Japan and cultured on potato-peptone-glucose

medium (Azegami *et al*., 1987) at 25 °C for 48 h. Bacteria were collected and suspended in sterile distilled water (DW). The concentrations of bacteria were adjusted to 10^7 cfu mL^{-1} with sterile DW for assessment of disease symptoms on rice seedlings treated with L-histidine or L-lysine. Bacterial suspensions were adjusted to 10^3 cfu mL^{-1} with sterile DW for assessment of disease symptoms on rice seedlings treated with 1-aminocyclopropane-1-carboxylic acid (ACC) or aminooxyacetic acid (AOA). These appropriate concentrations of bacteria for analysing the effects of L-histidine, L-lysine, ACC or AOA treatment on rice seedlings were determined by preliminary experiments (data not shown).

Treatment with L-histidine or L-lysine before or after inoculation with pathogenic *Burkholderia*

For treatment with L-histidine or L-lysine before inoculation with pathogenic *Burkholderia*, rice seeds were soaked for 48 h at 28 °C in 10 mM L-histidine or 10 mM L-lysine (Fig. S1). As a control, the seeds were soaked for 48 h at 28 °C in DW (Fig. S1). After soaking, the seeds were vacuum-infiltrated with 10^7 cfu mL^{-1} pathogenic *Burkholderia* for 5 min and then washed with DW three times. Vacuum-infiltration with *Burkholderia* was performed as described previously (Ando *et al*., 2014). A total of 25 *B. glumae* or *B. plantarii*-inoculated

seeds or control seeds were placed onto 150 g standardized commercial soil (Inaho; Inaho-kako Co.) in plastic pots (7.5 × 7.5 × 5.5 cm) containing 100 mL DW and overlaid with 5 mm depth of soil. Pots containing seeds were incubated in a plant growth chamber (CLE-303; Tomy Seiko Co.) at 30 °C for 48 h with a 14 h photoperiod under 171.43 μmol m^{-2} s^{-1} light (Fig. S1). After germination, the rice seedlings were cultivated a further 7 days under the same conditions, with regular irrigation with DW, before disease assessment (Fig. S1). The experiments were repeated three times.

For treatment with L-histidine or L-lysine after inoculation with pathogenic *Burkholderia*, rice seeds were soaked for 48 h at 28 °C in DW (Fig. S2). The seeds were vacuum-infiltrated with 10^7 cfu mL^{-1} pathogenic *Burkholderia* for 5 min and then washed with DW three times (Fig. S2). A total of 25 *B. glumae* or *B. plantarii*-inoculated seeds or control seeds were placed onto 150 g standardized commercial soil in plastic pots (7.5 × 7.5 × 5.5 cm) containing 100 mL of 10 mM L-histidine, 10 mM L-lsyine or DW as a control and overlaid with 5 mm depth of soil. These pots were incubated as described above for pre-inoculation treatments and disease was assessed 7 days after germination of the seedlings (Fig. S2). The experiments were repeated three times.

Treatment with ACC, an ethylene precursor, or AOA, an ACC synthase inhibitor

For treatment with ACC, an ethylene precursor, AOA, an ACC synthase (ACS) inhibitor or ACC plus AOA, rice seeds were soaked in 100 μM ACC, 100 μM AOA or 100 μM ACC plus 100 μM AOA for 48 h at 28 °C before inoculation with pathogenic *Burkholderia* (Fig. S3). As a negative control, rice seeds were soaked in DW for 48 h at 28 °C. The soaked seeds were vacuum-infiltrated with 10^3 cfu mL^{-1} pathogenic *Burkholderia* for 5 min and then washed with DW three times. Vacuum-infiltration with *Burkholderia* was performed as described previously (Ando *et al*., 2014). A total of 25 *B. glumae*-inoculated seeds or control seeds were placed onto 150 g standardized commercial soil in plastic pots (7.5 × 7.5 × 5.5 cm) containing 100 mL of 100 μM ACC, 100 μM AOA, 100 μM ACC plus 100 μM AOA or DW as a control and overlaid with 5 mm depth of soil. Pots containing seeds were incubated as described above for pre-inoculation treatments and disease was assessed 7 days after germination of the seedlings (Fig. S3).

As a positive control, rice seeds were soaked in DW for 48 h at 28 °C (Fig. S3). The soaked seeds were vacuum-infiltrated with 10^3 cfu mL^{-1} pathogenic *Burkholderia* for 5 min and washed with DW three times. A total of 25 *B. glumae*-inoculated seeds or control seeds were placed onto 150 g standardized commercial soil in plastic pots (7.5 × 7.5 × 5.5 cm)

containing 100 mL of a 10^3 cfu mL^{-1} suspension of the beneficial bacterium *Pseudomonas* sp. isolate W6, which was isolated from organic farming soil and can suppress rice bacterial seedling diseases through activation of the ET-dependent signalling pathway (Ando *et al*., 2014; authors' unpublished results). Seeds were overlaid with 5 mm depth of soil and incubated as described above for pre-inoculation treatments. Disease was assessed 7 days after germination of the seedlings (Fig. S3). All experiments were repeated three times.

Disease assessment of rice seedling diseases

The severity of the rice seedling blight caused by *B. glumae* as well as the rice seedling damping-off disease caused by *B. plantarii* were measured using a disease severity index (DSI) with a scale of 0 to 3, where 0 = healthy; 1 = growth suppression and chlorosis; 2 = partially dead; 3 = dead. Representative phenotypes of the scale 0 to 3 are shown in Figure S4. The DSI was calculated using the formula: $((1A + 2B + 3C)/3N) \times 100$, in which A = number of plants scored as 1; B = number of plants scored as 2; C = number of plants scored as 3; N = total number of plants (Ando *et al*., 2014). Statistical analysis of the disease severity indices for 25 rice seedlings planted in each type of soil was performed using the Steel–

Dwass test ($P < 0.01$). Photographs were taken of representative rice seedlings at 9 days after inoculation. The experiments were repeated three times and representative data are shown.

Measurement of ethylene production

After incubation of rice seeds in DW at 60 °C for 10 min to sterilize seed surfaces, 15 seeds were soaked in 10 mM L-histidine or DW as a control for 48 h at 28 °C in the dark. The germinated seeds were then placed on wet filter paper in a closed vial (2.5 cm diameter × 5 cm height) and incubated further at 28 °C in the dark. After 24 h, 1 mL of the gas in the closed vial was collected using a plastic syringe with a needle. The amount of ET produced was measured using gas chromatography as described previously (Hase *et al*., 2006) and the fresh weight of the germinated seedlings was measured. Five independent experiments were performed. The average amount (plus SD) of ET was calculated and compared between L-histidine and DW treatments using Student's *t*-test ($P < 0.05$).

Analysis of gene expression by quantitative RT-PCR

Expression of the ET-responsive defence-related gene, *OsGLP8*-12 and the ACC synthase 2 gene, *OsACS2*, was assessed by RT-PCR.

After surface sterilization of rice seeds, 10 seeds were soaked in 10 mM L-histidine or DW as a control for 48 h at 28 °C in the dark. After treatment, the seeds were incubated in DW at 28 °C in the dark for germination. The following molecular biological procedures were performed as described previously (Sambrook & Russell, 2001). RNA was isolated from each rice seedling using TRIzol RNA isolation regent (Thermo Fisher Scientific) according to the manufacturer's instructions. One microgram total RNA from each of five independent RNA samples from seedlings treated with L-histidine or DW was reverse transcribed into cDNA with random hexamer primers using a PrimeScript RT Reagent kit with gDNA Eraser (Takara-Bio) according to the manufacturer's instructions. Quantitative RT-PCR amplification was performed following the method described previously (Takahashi *et al*., 2014). Gene-specific primers for quantitative RT-PCR were as follows: germin-like F1 (5′-GCTAATTGATTGGCTCCAATC-3′) and germin-like R1 (5′-TAGCAACATATCGTGACACAC-3′) for *OsGLP8-12* (Yin *et al*., 2015); OsACS2-F (5′-GGAATAAAGCTGCTGCCGAT-3′) and OsACS2-R (5′-TGAGCCTGAAGTCGTTGAAGC-3′) for *OsACS2* (Shen *et al*., 2011); RUb-F1 (5′-

CCAGTAAGTCCTCAGCCATGGA-3′) and RUb-R1 (5′-GGACACAATGATTAGGGATCAC-3′) to amplify the ubiquitin gene as an internal control.

Results

Treatment of rice seeds with L-histidine suppresses bacterial seedling rot and seedling damping-off diseases and activates the expression of ET-responsive plant defence-related genes

Treatment of rice seeds with L-histidine before inoculation with *B. glumae* suppressed bacterial rot, whereas pretreatment with L-lysine or DW did not (Fig. 1). However, bacterial rot disease was not suppressed in rice seedlings that had been treated with L-histidine after vacuum-inoculation with *B. glumae* (Fig. 1). Similarly, treatment of rice seedlings with L-histidine before, but not after, vacuum-inoculation with *B. plantarii* also suppressed seedling damping-off disease caused by *B. plantarii*, whereas treatment with L-lysine or DW did not (Fig. 2). This observation suggests that pretreatment of the seedlings with L-histidine might activate the plant immune system.

Treatment of rice seeds with L-histidine accelerates ET production and induces ET-related gene expression

The amount of ET measured in the gas space of L-histidine-treated seedlings was higher than that detected from DW-treated seedlings (Fig. 3). Moreover, expression of *OsACS2*, which is associated with ET production, was induced in rice seedlings by L-histidine treatment (Fig. 4b). Expression of the ET-responsive defence-related gene, *OsGLP8-12*, was also up-regulated upon treatment with L-histidine but not with DW (Fig. 4a). These results suggest that exogenous application of L-histidine might activate ET production in rice seedlings.

Exogenous application of ACC suppresses bacterial rice seedling rot but AOA treatment compromises ACC-induced disease resistance

Treatment of rice seeds with ACC, an ethylene precursor, suppressed bacterial rice seedling rot disease caused by *B. glumae* as effectively as did treatment with L-histidine or the *Pseudomonas* sp. isolate W6 that is known to suppress rice seedling bacterial rot (Fig. 5).

In contrast, treatment of rice seeds with AOA, an inhibitor of ACC synthase, enhanced the development of symptoms of rice seedling rot disease (Fig. 5). Furthermore, in

seedlings treated with both ACC and AOA, the ACC-induced suppression of rice seedling rot disease was partially, although significantly, compromised by AOA (Fig. 5). Thus, ET production seems to be indispensable for suppressing rice seedling rot diseases caused by *B. glumae*.

Discussion

Abiotic and biotic compounds that can non-specifically induce resistance to a broad range of pathogens in plants have been characterized and released commercially in various countries (Lyon, 2014). The abiotic compound DL-3-aminobutyric acid (BABA), a non-proteinogenic amino acid, has great potential as a priming agent and exhibits suppressive activity against diseases caused by fungi, oomycetes, bacteria, viruses and nematodes (Balmer *et al.,* 2015; Baccelli & Mauch-Mani, 2016). Recently, the suppression of disease in plants treated with a proteinogenic amino acid has also been demonstrated (Kadotani *et al*., 2016; Seo *et al*., 2016). L-histidine induces resistance to *R. solanacearum* in tomato and *B. cinerea* in *A. thaliana* and induces the expression of a series of defence-related genes in *A. thaliana* (Seo *et al*., 2016). The present study has revealed the potential of L-histidine to suppress rice bacterial blight and seedling damping-off diseases. This observation suggests that L-histidine

might effectively suppress diseases in both dicotyledonous and monocotyledonous plants. In contrast, exogenous application of L-lysine did not suppress bacterial rice seedling diseases, even though L-lysine suppressed bacterial wilt disease caused by *R. solanacearum* in tomato (authors' unpublished results). Thus, the degree of induced resistance in response to L-lysine might differ between plant species.

L-histidine was shown to induce resistance to *R. solanacearum* and *B. cinerea* through the activation of ET signalling in tomato and *A. thaliana* (Seo *et al*., 2016). In the present study, the expression of ET-responsive *OsGLP8-12* and ET biosynthesis-related *OsACS2* was enhanced by exogenous application of L-histidine to germinated rice seeds. In general, activation of the plant defence system against pathogen infection is modulated by plant hormones, such as salicylic acid (SA), jasmonic acid (JA), ET and abscisic acid (ABA) (Robert-Seilaniantz *et al*., 2011; Pieterse *et al*., 2012; Vos *et al*., 2013). Germinated rice seedlings exogenously treated with L-histidine released more ET than did DW-treated control seedlings. These results suggest that the suppression of bacterial rice seedling diseases might require ET signalling. This hypothesis seems to be supported by the fact that treatment of germinated rice seeds with ACC, an ethylene precursor, could suppress bacterial rice seedling rot disease as effectively as treatment with L-histidine, whereas treatment of rice seeds with

AOA, an inhibitor of ACS, increased disease symptom severity. ET has been described as playing a key role during signal transduction that leads to the induction of the plant defence system against various pathogens (Hase *et al*., 2006; Iwai *et al*., 2006; van Loon *et al*., 2006; Seo *et al*., 2012; Yin *et al*., 2015). Moreover, the timing of ET application appears to be critical for induced resistance, as pretreatment of seedlings with ET or ACC before inoculation with pathogens can increase resistance, while treatment with these compounds after inoculation does not (van Loon *et al*., 2006). Similarly, in the present investigation, bacterial diseases were suppressed by treatment of germinated rice seeds and seedlings with L-histidine before, but not after, inoculation with pathogenic *Burkholderia*. The similar effects of L-histidine and ET on suppression of bacterial rice seedling diseases seems to further support the hypothesis of a connection between ET-mediated signalling and L-histidine -induced disease resistance.

The management of rice nurseries involves risks of infection of germinated seeds and seedlings by seedborne pathogens such as *B. glumae* and *B. plantarii* (Uematsu *et al*., 1976; Azegami *et al.*, 1987). Not only may seeds be already infected with pathogens, but the incubation of germinating seeds at high humidity and growth of rice seedling plants in centralized greenhouses provide further opportunities for infection. Thus, infection with

pathogenic *Burkholderia* could occur during any of the multiple processes of rice nursery management, from seed disinfection through to raising seedlings. For this reason, the results of this study, which indicate the disease-suppressive activity of L-histidine in rice seedlings, support the expanded use of AgrevoEX to control rice seedling diseases in rice production systems.

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Supporting Information

Additional Supporting Information may be found in the online version of this article at the publisher's web-site.

Figure S1 Experimental procedure for treatment of rice seeds with L-histidine, L-lysine, or distilled water (DW; control) before inoculation with *Burkholderia glumae* and *B. plantarii*.

Figure S2 Experimental procedure for treatment of rice seeds with L-histidine, L-lysine, or distilled water (DW; control) after inoculation with *Burkholderia glumae* and *B. plantarii*.

Figure S3 Experimental procedure for treatment of rice seeds with 1-aminocyclopropane-1-carboxylic acid (ACC), an ethylene precursor, or aminooxyacetic acid (AOA), an ACC synthase (ACS) inhibitor, before inoculation with *Burkholderia glumae*. Treatment with distilled water (DW) or *Pseudomonas* sp. isolate W6 acted as negative and positive controls, respectively.

Figure S4 Representative phenotypes of each category on the disease scale of rice seedling blight caused by *Burkholderia glumae*. The severity of the rice seedling blight caused by *B. glumae* as well as the rice seedling damping-off disease caused by *B. plantarii* were measured using a disease severity index (DSI), which was assessed using a scale of 0 to 3, where 0 = healthy; 1 = growth suppression and chlorosis; 2 = partially dead; 3 = dead. The bar indicates 3 cm.

Figure legends

Figure 1 Effect of exogenous application of L-histidine (His), L-lysine (Lys), or distilled water (DW; control) on development of bacterial seedling rot disease caused by *Burkholderia glumae*. A total of 25 seedlings per treatment were tested. (a) Representative development of bacterial rot symptoms in rice seedlings treated with 10 mM L-histidine, 10 mM L-lysine, or DW before (e.g. +His/B) or after (e.g. B/+His) vacuum-inoculation with *B. glumae*. (b) Severity of bacterial rot disease in treated and control rice seedlings. Disease severity was assessed on a scale of 0 to 3 from uninfected to severely infected. The percentage of seedlings in each category (0, 1, 2 or 3) among the 25 seedlings in each treatment is indicated by open, hatched, grey or closed bars, respectively. The disease severity indices (DSI) of

seedlings in each experiment (n = 25) are shown above each bar. Statistically significant differences were identified using the Steel–Dwass test ($P < 0.01$) and are indicated by different letters.

Figure 2 Effect of exogenous application of L-histidine (His), L-lysine (Lys) or distilled water (DW; control) on bacterial seedling damping-off disease caused by *Burkholderia plantarii*. A total of 25 seedlings per each treatment were tested. (a) Representative development of rice bacterial damping-off disease symptoms in rice seedlings germinated from seeds treated with 10 mM L-histidine, 10 mM L-lysine, or DW before (e.g. +His/B) or after (e.g. B/+His) vacuum-inoculation with *B. plantarii*. (b) Severity of bacterial damping-off disease in treated and control rice seedlings. Disease severity was assessed on a scale of 0 to 3 from uninfected to severely infected. The percentage of seedlings in each category (0, 1, 2 or 3) among the 25 seedlings in each treatment is indicated by open, hatched, grey or closed bars, respectively. The disease severity indices (DSI) of seedlings grown in each treatment (n = 25) are shown above each bar. Statistically significant differences were identified using the Steel–Dwass test ($P < 0.01$) and are indicated by different letters.

Figure 3 Ethylene (ET) production from rice seeds treated with L-histidine (His) or distilled water (DW; control). Fifteen seeds were used for each treatment and the experiments

were repeated five times. The average amount (plus standard deviation) of ethylene produced was calculated, and treatments were found to be statistically different (indicated by asterisk) using Student's *t*-test ($n = 5$, *t*-test $P < 0.05$).

Figure 4 Expression of ethylene-responsive defence-related gene, *OsGLP8-12*, and ACC synthase 2 gene, *OsACS2*, in rice seedlings treated with L-histidine or distilled water (DW) as a control. The abundances of *OsGLP8-12* (a) and *OsACS2* (b) transcripts relative to transcripts of the internal standard ubiquitin in rice seedlings treated with L-histidine or DW were measured by quantitative RT-PCR.

Figure 5 Suppression of bacterial rice seedling rot disease by 1-aminocyclopropane-1-carboxylic acid (ACC) treatment and compromise of ACC-induced disease resistance by aminooxyacetic acid (AOA) treatment. Bacterial rot disease symptoms in rice seedlings treated with ACC, AOA, ACC plus AOA, distilled water (DW) as a negative control, or *Pseudomonas* sp. isolate W6 as positive control were assessed. A total of 25 seedlings per treatment were tested. (a) Representative development of rice bacterial rot disease symptoms in rice seedlings germinated from seeds treated with 100 μM ACC, 100 μM AOA, ACC plus AOA, or DW. (b) Severity of bacterial rot disease in treated rice seedlings was assessed on a scale of 0 to 3 from uninfected to severely infected seedlings. The percentage of seedlings in

each disease severity category (0, 1, 2 or 3) among 25 seedlings of each experiment is indicated by open, hatched, grey or closed bars, respectively. The disease severity indices (DSI) of seedlings grown in each treatment (n = 25) are shown above each bar. Statistically significant differences were identified using the Steel–Dwass test ($P < 0.01$) and are indicated by different letters.